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(54) Title: **INCREASING PLANT YIELD BY SEED TREATMENT WITH A NEONICOTINOID COMPOUND**

(57) Abstract: The yield and/or the vigor of an agronomic plant can be increased or improved in locations where the level of insect infestation is below that indicating the need for the use of an insecticide for insect control purposes by treating a seed of the plant with a neonicotinoid compound. The method is useful for non-transgenic plants and for plants having a foreign gene that encodes for the production of a modified *Bacillus thuringiensis* delta-endotoxin protein. A method of improving the results of a plant breeding program, a method of marketing plant seed, and a seed that has been treated by the method are also described.

**INCREASING PLANT YIELD AND/OR VIGOR BY SEED TREATMENT  
WITH A NEONICOTINOID COMPOUND**

**BACKGROUND OF THE INVENTION**

**(1) Field of the Invention:**

5     **[0001]**     The present invention relates to the improvement of the yield and/or the vigor of agronomic plants, and more particularly to a method of improving the yield and/or vigor of agronomic plants by treatment of a seed of the plant with a neonicotinoid compound when insecticidal protection is not indicated.

10    **(2) Description of the Related Art:**

**[0002]**     Plants are a critical source of food, animal feed, fiber, lumber, structural materials, and useful chemicals and medicaments. Increasing demands for these plant products have driven continuing worldwide efforts to increase the productivity of arable lands. These efforts have resulted in  
15    large increases in land productivity and crop yield. Most of these increases can be attributed to improved plant varieties and increased use of pesticides, new types of pesticides with higher activities, new types of herbicides and increased use of herbicides, and the continued use of fertilizers.

20    **[0003]**     In contrast to the benefits provided by these factors, however, each of them has disadvantages. For example, higher yielding varieties of crops can be less robust and may be subject to catastrophic loss to pests or environmental stresses to which they are not acclimated; the toxic activities of pesticides and herbicides are often not limited to pests and  
25    can be harmful to non-target species - including humans; and fertilizers can be lost by leaching and runoff to surface waters and cause serious disruption of natural stream life and water quality.

**[0004]**     New types of pesticides have been discovered that are very effective against targeted pests. One family of insecticides, in particular,  
30    has been found that shows great potential for protecting the seeds and plants of important agronomic crops from insect damage. This family, the neonicotinoids, include such agents as thiamethoxam (available

commercially as HELIX® and CRUISER®), imidacloprid (available commercially as GAUCHO®), as well as several other related compounds. The use of thiamethoxam as a pesticidal seed treatment has been reported, at least on cotton, sorghum, maize, sweet corn, and sugar beet, for the control of wireworm, cotton seedling thrips, tomato thrips, cotton aphid, black field earwig, and other insects. Seed treatment with imidacloprid has been reported, at least for winter cereals, corn, wheat, barley, sugar beets, sorghum, potato, cotton and canola, for the control of aphids, flea beetles, Lygus bugs, cabbage Seedpod Weevil larvae, corn root worm, chinch bug, wireworms, and other insect pests. The use of these insecticides as seed treatments, rather than as field-applied formulations, is believed to reduce the exposure and odor of the pesticide, and to reduce the amount of post-planting cultivation and application. For further information, see, e.g., U.S. Patent No. 6,331,531 B1, and WO 99/35913.

[0005] Another area of agricultural pest control in which significant progress has been made is with the genetic engineering of plants to express insecticidally toxic proteins, in particular, the delta endotoxins of *Bacillus thuringiensis* (Bt). A comprehensive listing of such Bt endotoxins can be found, for example, at [http://epunix.biols.susx.ac.uk/Home/Neil\\_Crickmore/Bt/index.html](http://epunix.biols.susx.ac.uk/Home/Neil_Crickmore/Bt/index.html); on 04/27/2002.

[0006] Several reports have discussed the combination of treating transgenic plants that produce insect toxins with pesticidal compounds for the purpose of insect control. For example, Lee, B. *et al.*, in WO 99/35913, describe a method of controlling pests by treating plants that express one or more naturally occurring Bt insect toxins with a neonicotinoid compound. In WO 99/35910, a method of controlling pests is described that includes applying pymetrozine, profenofos, a benzoylurea-derivative, or a carbamate-derivative to the pests, their environment, or to a transgenic plant that can contain one or more of the natural Bt delta-endotoxin genes. In U.S. Patent No. 6,331,531, Kern

describes the treatment of transgenic crops with certain compounds, including imidacloprid, in order to obtain synergistic control of harmful insects. Commercially, Monsanto Company, St. Louis, MO, has offered GAUCHO®-treated corn that is Roundup Ready® (hybrids RX738RR and RX740RR), corn that has YieldGuard® corn borer (hybrid DK626BtY), and corn that has both Roundup Ready® and YieldGuard® corn borer transgenic events (hybrids DK440RR/YG, DK520RR/YG, DK551RR/YG, and RX601RR/YG). The purpose of applying the insecticide to the seed is described as being for protection to the first true leaf stage against pests like wireworms, seed corn maggots, imported fire ants, and flea beetles.

[0007] With the continued development of molecular cloning techniques, various delta-endotoxin genes have been isolated and their DNA sequences determined. These genes have been used to construct certain genetically engineered Bt products that have been approved for commercial use. Recent developments have seen new delta-endotoxin delivery systems developed, including plants that contain and express genetically engineered delta-endotoxin genes.

[0008] The cloning and sequencing of a number of delta-endotoxin genes from a variety of Bt strains have been described and are summarized by Hofte and Whiteley, *Microbiol. R.*, 53:242-255 (1989). Plasmid shuttle vectors designed for the cloning and expression of delta-endotoxin genes in *E. coli* or *B. thuringiensis* are described by Gawron-Burke and Baum, *Genet. Engineer*, 13:237-263 (1991). U.S. Pat. No. 5,441,884 discloses a site-specific recombination system for constructing recombinant *B. thuringiensis* strains containing delta-endotoxin genes that are free of DNA not native to *B. thuringiensis*.

[0009] In recent years, researchers have focused effort on the construction of hybrid delta-endotoxins with the hope of producing proteins with enhanced activity or improved properties. Advances in the art of molecular genetics over the past decade have facilitated a logical and orderly approach to engineering proteins with improved properties. Site-specific and random mutagenesis methods, the advent of polymerase

chain reaction (PCR™) methodologies, and the development of recombinant methods for generating gene fusions and constructing chimeric proteins have facilitated an assortment of methods for changing amino acid sequences of proteins, fusing portions of two or more proteins together in a single recombinant protein, and altering genetic sequences that encode proteins of commercial interest.

**[00010]** However, in earlier work with crystal proteins, these techniques were only exploited in limited fashion. The likelihood of arbitrarily creating a chimeric protein with enhanced properties from portions of the numerous native proteins which have been identified was remote given the complex nature of protein structure, folding, oligomerization, activation, and correct processing of the chimeric protoxin to an active moiety. Only by careful selection of specific target regions within each protein, and subsequent protein engineering can toxins be synthesized which have improved insecticidal activity.

**[00011]** In U.S. Patent No. 6,281,016, however, English *et al.* disclosed reliable methods and compositions comprising recombinantly-engineered crystal proteins which have improved insecticidal activity, broad-host-range specificities, and which are suitable for commercial production in *B. thuringiensis*. That work describes methods for the construction of *B. thuringiensis* hybrid delta-endotoxins comprising amino acid sequences from native Cry1Ac and Cry1F crystal proteins. These hybrid proteins, in which all or a portion of Cry1Ac domain 2, all or a portion of Cry1Ac domain 3, and all or a portion of the Cry1Ac protoxin segment is replaced by the corresponding portions of Cry1F, possess not only the insecticidal characteristics of the parent delta-endotoxins, but also have the unexpected and remarkable properties of enhanced broad-range specificity which is not proficiently displayed by either of the native delta-endotoxins from which the chimeric proteins were engineered.

**[00012]** One method of using genes which encode insect toxins is to incorporate the gene into the plant requiring protection. Techniques for carrying out this transformation are known in the art, and can be found in,

for example, U.S. Patent Nos. 6,023,013 and 6,284,949, among others. In commercial practice, it is common to transfer desired insecticidal toxin genes into genetic stock of the agronomic plant that is stable and vigorous, but is not the top yielding variety. Once the transgenic event is stabilized  
5 in the selected recipient, a normal hybrid breeding and selection process is used to cross the transgenic plants with higher-yielding varieties in order to obtain high-yielding varieties that express the desired transgenic event. Finally, when a hybrid is selected that demonstrates suitable yield and vigor, while also expressing the transgenic event, it can proceed to  
10 commercial use.

**[00013]** A disadvantage to this technique which remains, however, is that it is not uncommon for hybrid varieties of the plant, and, in particular, for transgenic hybrids, to demonstrate lower vigor, such as, for example, less vigorous root growth and development, than parent and non-  
15 transgenic varieties.

**[00014]** Therefore, even with such advances as described above, the demand continues for increased productivity from useful agricultural land, irrespective of whether these increases are due to pest control or to other factors. Accordingly, it remains a high priority to provide methods for  
20 increasing the yield and vigor of agronomic plants. It would be useful if these methods were safe and easy to use. Moreover, it would be useful if these methods could help reduce the amount of in-field cultivation and chemical application to plants during growth. It would also be useful if these methods could be carried out with reduced exposure of farmers and  
25 surrounding land and water, and non-target plants and animals to toxic pesticides. It would also be useful if these methods could be used in beneficial combination with other emerging technologies, such as to enhance the vigor of hybrid and, in particular, transgenic hybrid plants that express insecticidal toxins.

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## SUMMARY OF THE INVENTION

[00015] Briefly therefore, the present invention is directed to a novel method of increasing the yield and/or vigor of an agronomic plant that is grown from a seed, the method comprising:

- 5           a.     determining whether the seed is to be planted in a location having a level of insect pest infestation that would indicate treatment with an insecticide; and, if such treatment is not indicated,
- b.     carrying out an action that is selected from the group consisting of:
- 10           i.     treating the seed with a neonicotinoid compound,
- ii.    recommending the purchase of a seed that has been treated with a neonicotinoid compound for planting in the location,
- iii.   selling a seed that has been treated with a neonicotinoid compound for planting in the location, and
- 15           iv.    planting in the location a seed that has been treated with a neonicotinoid compound.

[00016] The present invention is also directed to a novel method of increasing the yield and/or vigor of an agronomic plant that is grown from a seed that is planted in a location having a level of infestation by an insect

20           that is a pest for the agronomic plant and against which a neonicotinoid compound has insecticidal activity, the method comprising:

- a.     determining whether the level of infestation by the insect that is a pest for the agronomic plant indicates treatment with an insecticide; and, if treatment is not indicated,
- 25           b.     treating the seed with a neonicotinoid compound.

The present invention is also directed to a novel method of breeding a hybrid plant having increased yield and/or vigor from two parent plants, the method comprising:

- treating the seeds of one or both of the parent plants with a
- 30           neonicotinoid compound prior to planting the seeds;
- pollinating the female parent with pollen of the male parent; and
- gathering the seed produced by the female parent plant.

[00017] The present invention is also directed to a novel method of increasing the yield and/or vigor of an agronomic plant that is grown from a seed that is planted in a location where treatment of the seed or the agronomic plant with an insecticide is not indicated, the method comprising treating a seed with a neonicotinoid compound and planting the treated seed in a location where treatment of the seed or the agronomic plant with an insecticide is not practiced.

[00018] The present invention is also directed to a novel method of increasing the yield and/or vigor of an agronomic plant that is grown from a seed that is planted in a location having a level of infestation by an insect that is a pest for the agronomic plant and against which a neonicotinoid insecticide has insecticidal activity, the method comprising treating a seed with a neonicotinoid compound and planting the treated seed in a location where insecticide treatment of the seed or the agronomic plant is not practiced.

[00019] The present invention is also directed to a novel method of increasing the yield and/or vigor of an agronomic plant that is grown from a seed that is planted in a location having a level of infestation by an insect that is a pest for the agronomic plant and against which a neonicotinoid insecticide has insecticidal activity, the method comprising:

- a. treating a seed with a neonicotinoid insecticide; and
- b. planting the treated seed in a location having a level of insect infestation below that at which such insecticide treatment is indicated.

[00020] The present invention is also directed to a novel method of marketing plant seed that are treated with a neonicotinoid compound to provide an increase in the yield and/or vigor of an agronomic plant that is grown from the seed, the method comprising:

- a. determining whether the seed is to be planted in a location having a level of insect infestation that indicates a need for such treatment, and, if not;
- b. carrying out an action selected from the group consisting of:



- i. recommending that such treated seed be purchased and planted,
- ii. advertising such treated seed,
- iii. obtaining such treated seed for resale, and
- 5. iv. selling such treated seed.

**[00021]** The present invention is also directed to a novel method of increasing the yield and/or vigor of an agronomic plant that is grown from a seed, the method comprising:

- a. selecting a location in which the seed is to be planted where  
10 the level of insect pest infestation is below that at which treatment with an insecticide is indicated; and
- b. carrying out an action that is selected from the group consisting of:
  - i. treating the seed with a neonicotinoid compound,
  - 15 ii. recommending the purchase of a seed that has been treated with a neonicotinoid compound for planting in the location,
  - iii. selling a seed that has been treated with a neonicotinoid compound for planting in the location, and
  - iv. planting in the location a seed that has been treated  
20 with a neonicotinoid compound.

**[00022]** The present invention is also directed to a novel seed that is treated by the method described first above.

**[00023]** Among the several advantages found to be achieved by the present invention, therefore, may be noted the provision of a method of  
25 increasing the yield and vigor of agronomic plants, and also the provision of such methods that are safe and easy to use, and also the provision of such methods that can help reduce the amount of in-field cultivation and chemical application to plants during growth, and also the provision of such methods that can be carried out with reduced exposure of farmers  
30 and surrounding land and water, and non-target plants and animals to toxic pesticides, and also the provision of methods that can be used in beneficial combination with other emerging technologies, such as to

enhance the vigor of hybrid, and in particular, transgenic hybrid plants that express insecticidal toxins.

#### BRIEF DESCRIPTION OF THE DRAWINGS

[00024] Figure 1 is a map illustrating levels of insecticide use on corn acreage in the United States in the year 2001 by crop reporting district;

[00025] Figure 2 is a bar chart showing the corn yield (in bu/ac) from seed having a seed treatment with imidacloprid (GAUCHO®) relative to the yield of control corn without such seed treatment for twelve different corn hybrids; and

[00026] Figure 3 is a bar chart showing the corn yield (in bu/ac) from seed having a seed treatment with imidacloprid (GAUCHO®) relative to the yield of control corn without such seed treatment for twenty-four different locations.

#### DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[00027] In accordance with the present invention, it has been discovered that the vigor and/or the yield of an agronomic plant can be increased by treating the seed of the plant with an effective amount of a neonicotinoid compound of the type that has heretofore been principally identified as an insecticide. Surprisingly, it has been shown that such neonicotinoid compounds have the capability of causing an improvement in the yield and/or the vigor of the plant whether or not the plant is under pest pressure from insect pathogens. In fact, the increase in yield and/or vigor can be shown to take place even when the treated seed and plant are under no pest pressure at all, for example, as in tests where germination, sprouting and plant growth take place under substantially sterile conditions.

[00028] The increase in yield and/or vigor is entirely unexpected because it is brought about by the use of a compound that has previously been identified as an insecticide, but occurs even in the absence of pest pressure by insect pathogens against which the compound is known to be active. By way of example, the method is useful to increase plant yield and/or vigor in geographic areas, or with cultivation practices, where the

particular insecticide is not normally used -- and even under conditions where the use of the insecticide is explicitly not indicated.

[00029] In fact, it is believed that it would be counterintuitive for someone having skill in the art of controlling insect pathogens in crops to apply a chemical compound to a seed or a plant -- at significant expense -- in instances where the known activity of the compound was believed not to be needed. Moreover, given the care expended upon minimizing the use of resources in modern farming practices, such an application would be considered to be a waste. But, surprisingly, the inventors have found that this is not the case. The inventors have found that some neonicotinoid compounds -- neonicotinoid insecticides, in particular -- can be applied to plant seeds with the result that the plants that are grown from the seeds demonstrate increased yield and/or vigor.

[00030] It is also believed that the novel method demonstrates particularly useful and unexpected results in situations where the treated seed or plant is subjected to some stress during or after germination. For example, such stress could be caused by environmental stress, such as drought, cold, cold and wet, and other such conditions. It is believed, in fact, that side-by-side comparisons of plants grown from seeds treated by preferred embodiments of the novel method and plants grown from untreated seeds are subjected to drought conditions sometime after sprouting will demonstrate the superior yield and/or vigor of the plants grown from the treated seeds.

[00031] Since the neonicotinoid compounds that are useful in the novel method can be applied to seed prior to planting, the present method provides an easy method of achieving the advantages of improved plant yield and/or vigor without the added effort and expense of cultivation or in-field application after germination and sprouting.

[00032] In another embodiment, the neonicotinoid compound can be applied with good results to the seeds of plants having particular transgenic events, whether or not insect infestation level indicates the use of an insecticide. In one example of this embodiment, the neonicotinoid

compound is applied to a seed that contains one or more genes capable of expressing a *B. thuringiensis* delta-endotoxin of any type, when such neonicotinoid treatment is not indicated on account of insect pressure. In another example of this embodiment, the neonicotinoid compound is

5 applied to a seed that contains one or more genes capable of expressing a chimeric or modified Bt delta-endotoxin, which has an amino acid sequence that is different from that of any natural, unmodified, endotoxin, such as those described in WO 99/35910 and WO 99/35913. An unexpected advantage of the treatment of the seed of a transgenic plant is

10 the surprising increase in vigor that the method provides to the transgenic plant. In preferred embodiments, the combination of neonicotinoid seed treatment with a transgenic plant provides a synergistic advantage. This is of particular value, for example, in breeding programs for transgenic plants.

15 **[00033]** As mentioned above, the application of the neonicotinoid compound has the capability of increasing the yield and/or vigor of a plant even in the absence of insect pests against which the compound has insecticidal activity. In fact, the neonicotinoid compound is capable of increasing the yield and/or the vigor of a plant even when the seed is

20 germinated and sprouted and the plant is grown under sterile conditions. In other words, in the absence of any plant pests at all.

**[00034]** When it is said that the seed is germinated and sprouted and the plant is grown under sterile conditions, what is meant is that a seed, which has been subjected to a non-phytotoxic surface sterilization

25 procedure, such as contact with 0.1% - 0.15% sodium hypochlorite solution containing 0.5% household detergent for 10 minutes, followed with rinsing 3 times with sterile distilled water, or to other appropriate sanitization procedures as are known in the art, is planted in a growing medium that has been sterilized, or is otherwise substantially free of insect

30 pests and other organisms that are pathogenic for the plant.

**[00035]** Unless otherwise indicated, when an "insect pest", or an "insect that is a pest for the agronomic plant", is referred to, what is meant is an

insect species known to be an important pest of a particular agronomic plant. A pest would normally be considered to be an important pest of a particular plant or crop if that pest was capable of reducing the yield and/or the vigor of the plant or crop to a level below that which the plant or crop would provide in the absence of the pest.

**[00036]** As used herein, the terms "agronomic plant" and "agronomically important plant" mean the same thing, and both refer to a plant of which a part or all is, or has been, harvested or cultivated on a commercial scale, or serves as an important source of feed, food, fiber, lumber, or other chemical compounds. Examples of such agronomic plants include, without limitation, corn, cereals, including wheat, barley, rye, and rice, vegetables, clovers, legumes, including beans, peas and alfalfa, sugar cane, sugar beets, tobacco, cotton, rapeseed (canola), sunflower, safflower, and sorghum. Other agronomic plants will be described below.

**[00037]** When the subject method is described herein as "increasing the yield" of an agronomic plant, what is meant is that the yield of a product of the plant is increased by a measurable amount over the yield of the same product of the plant produced under the same conditions, but without the application of the subject method. It is preferred that the yield be increased by at least about 0.5%, more preferred that the increase be at least about 1%, even more preferred is about 2%, and yet more preferred is about 4%, or more. Yield can be expressed in terms of an amount by weight or volume of a product of the plant on some basis. The basis can be expressed in terms of time, growing area, weight of plants produced, amount of a raw material used, or the like. By way of example, if untreated soybeans yielded 35 bu/ac, and if soybeans that received the subject treatment yielded 38 bu/ac under the same growing conditions, then the yield of soybeans would be said to have been increased by  $((38-35)/35) \times 100 = 8.5\%$ . This increase in yield would be considered to be within the definition of "increasing the yield" of soybeans as those terms are used herein.

[00038] In the same manner, if a particular desired component of an agronomic plant is increased by a measurable amount over the yield of the same component of the plant produced under the same conditions, but without the application of the subject method, then the yield of the agronomic plant is increased. By way of example, if untreated soybeans (weighing 60 lb/bu) yielded 35 bu/ac of beans having an oil content of 20% by weight, and if soybeans that received the subject treatment yielded 35 bu/ac of beans having an oil content of 22% by weight under the same growing conditions, then the yield of soybean oil would be said to have been increased by  $((0.22 \cdot 60 \cdot 35) - (0.2 \cdot 60 \cdot 35)) / (0.2 \cdot 60 \cdot 35) \times 100 = 10\%$ . This increase in oil yield would be considered to be within the definition of "increasing the yield" of an agronomic crop as those terms are used herein.

[00039] When the subject method is described herein as "increasing the vigor" of an agronomic plant, what is meant is that the vigor rating, or the stand (the number of plants per unit of area), or the plant weight, or the plant height, or the plant canopy, or the visual appearance, or the root rating, or any combination of these factors, is increased or improved by a measurable or noticeable amount over the same factor of the plant produced under the same conditions, but without the application of the subject method. It is preferred that such factor(s) is increased or improved by a significant amount.

[00040] When it is said that the present method is capable of "increasing the yield and/or vigor" of an agronomic plant, it is meant that the method results in an increase in either the yield, as described above, or the vigor of the plant, as described above, or both the yield and the vigor of the plant.

[00041] As used herein, the term "location" means the place where the seed is planted, and when the seed is planted in a field, garden or seedbed, it includes the geographic area around the field, garden or seedbed that would be expected to have the same level of insect pest infestation as the place where a seed is planted. By way of example,

adjacent fields and fields located within reasonable proximity to the place where a seed is planted would normally be expected to have the same level of insect pest infestation. In some cases, an entire growing region, such as a county, or several counties, or a crop reporting district, or even a state, or larger region, would be expected to have the same level of insect pest infestation. It is believed that the delineation of such regions, and methods for determining their extent, are common knowledge within the skill of an ordinary practitioner in the art of agricultural pest control.

[00042] The terms "level of infestation", as used herein, mean the capacity for plant damage by the infesting entity expressed on some basis. The basis can be per unit area, per unit time, per plant, or the like. In the present case, a level of infestation can include zero infestation. Common parameters for the level of infestation of insects include, for example, the concentration of the insects in terms of number per unit area, and the number of insects found, caught, or otherwise counted, per unit time in a specific location.

[00043] When it is said that an insect is one "against which a neonicotinoid compound has insecticidal activity", it is meant that a neonicotinoid insecticide, such as imidacloprid, thiamethoxam, or clothianidin, for example, has a toxic effect against the insect. Such toxic effect can include direct or indirect actions such as inducing the death of the insect, repelling the insect from the plant seeds, roots, shoots and/or foliage, inhibiting feeding of the insect or its larval stages on, or the laying of its eggs on, the plant seeds, roots, shoots and/or foliage, and inhibiting or preventing reproduction of the insect.

[00044] In an embodiment of the subject method the yield and/or the vigor of an agronomic plant that is grown from a seed can be increased by determining whether the seed is to be planted in a location having a level of insect pest infestation that would indicate treatment with an insecticide; and, if such treatment is not indicated, carrying out an action that is selected from the group consisting of: (i) treating the seed with a neonicotinoid compound, (ii) recommending the purchase of a seed that

has been treated with a neonicotinoid compound for planting in the location, (iii) selling a seed that has been treated with a neonicotinoid compound for planting in the location, or (iv) planting in the location a seed that has been treated with a neonicotinoid compound.

5     **[00045]**     In an alternative embodiment, the method can be carried out by selecting a location in which the seed is to be planted where the level of insect pest infestation is below that at which treatment with an insecticide is indicated; and carrying out an action that is selected from the group consisting of: (i) treating the seed with a neonicotinoid compound, (ii)  
10     recommending the purchase of a seed that has been treated with a neonicotinoid compound for planting in the location, (iii) selling a seed that has been treated with a neonicotinoid compound for planting in the location, or (iv) planting in the location a seed that has been treated with a neonicotinoid compound.

15     **[00046]**     Surprisingly, the present method requires one to do precisely what the present state of knowledge in pesticide practice would teach one not to do -- to treat the seed with a neonicotinoid compound known heretofore as an insecticide -- when the use of an insecticide is not indicated.

20     **[00047]**     The determination of whether the level of infestation by the insect that is a pest for the agronomic plant indicates treatment with an insecticide can be made in any one of several ways and is a determination that is well known to one having ordinary skill in the art of pest control. By way of example, one method for making this determination is to compare  
25     the yield or vigor of the agronomic plant when it is grown in the location without any insecticide treatment (for example, as an untreated control) with the yield or vigor of the plant when it is grown in the same location with a standard soil treatment of insecticide. If the soil treatment with the insecticide does not result in improvement of the yield or vigor of the plant,  
30     this would be considered to be a determination that treatment with an insecticide was not indicated. In making this determination, it is preferred that the soil-applied insecticide is a neonicotinoid insecticide.



**[00048]** Another method for determining that treatment with an insecticide is not indicated is to review historical data for a particular location, and, if seeds of the agronomic plant have not historically been treated with an insecticide at that location -- even when such seed treatment was approved for use and was commercially available -- then it can be determined that such a treatment was not indicated.

**[00049]** An example of a method for determining that treatment with an insecticide is not indicated by reviewing historical data for a particular location is exemplified by reference to pertinent data showing actual insecticide treatment patterns for a selected crop. In the United States, for example, certain crop reporting districts (CRDs) have been defined, which delineate geographical areas within which growing conditions are the same or similar. Data is historically compiled for each of these CRDs on the types and acreage of crops planted, as well as for insecticide usage. Commercial companies that serve the agricultural sector, such as Doane Market Research, Doane Agricultural Services, Inc., St. Louis, MO, provide such information. By way of example, data showing planted acreage, acreage treated with insecticide, acreage treated with foliar insecticide, acreage treated with soil applied insecticide, and acreage that is not treated with insecticide, can be provided for crops such as corn, cotton, and soybeans. Inspection of this information by a skilled practitioner would readily permit the determination of whether treatment of the pertinent crop was indicated for a particular location. In particular, treatment would not be indicated for a CRD, or other reporting region, in which no insecticide treatment is shown. In fact, without knowledge that treatment would provide benefits of yield and/or vigor in a manner other than as a pesticide, the election to use an insecticide in a location where no insecticide use is reported would be counterintuitive.

**[00050]** Because information on infestation and insecticide usage is commonly available to seed companies, seed distributors and sellers, and farmers, it must be assumed that this information is well known to the pertinent public. Therefore, seed treatment would not be indicated for any

location where treatment is not practiced. As used herein, the terms "treatment is not practiced", as they modify a location or region where crops are planted, means that under 1% of the total acreage planted to a crop have been reported as being treated. A preferred level of determining where treatment is not practiced is that 0.5%, or under, of the total acreage planted to a crop is treated, even more preferred that under 0.1% of the total acreage planted to a crop is treated, and yet more preferred that none of the total acreage planted to a crop is treated.

[00051] Maps and tables can be provided that show the locations where the insecticidal treatment of corn is not practiced. It is believed, therefore, that seed treatment of corn with a neonicotinoid compound having insecticidal properties would not be indicated for those locations. Similar data can be shown for cotton and soybeans, among other crops, and this data can serve, likewise, as the basis for indicating that seed treatment of these crops with a neonicotinoid compound having insecticidal properties is not indicated.

[00052] By way of example, data is available that shows the total acreage in each CRD that is planted to a particular crop in the U.S., and how many, and which, of those acres receive insecticide treatment. For corn in the U.S., for example, Table 1 shows that of the about 76 million acres that were planted to corn, only about 21 million acres, or less than 30% of the total acreage, received an insecticide treatment. That means that over 70% of the corn acreage received no insecticide treatment.

Figure 1 shows this same information in a graphic format. (Source of Fig.1: 2001 Doane AgroTrak Study -- Doane Marketing Research, Inc., St. Louis, Missouri). Because insecticides approved for corn, and corn seeds treated with insecticides, were approved for use and were readily available on the market, It is believed that such insecticides and insecticidally treated seeds would have been used in locations where their use would have been economically justified. It is believed that non-use in a particular location, therefore, would indicate a level of insect infestation at that location below that indicating insecticide treatment.

Table 1: Corn acreage that is treated and untreated with insecticide by U.S. crop reporting district in 2001.

CRD Numeric	Planted	Treated Base Acres	Soil Treated Base Acres	Foliar Treated Base Acres	Non Treated Base Acres
01010	36,638				36,638
01020	46,741				46,741
01030	17,041				17,041
01040	30,183	2,040	2,040	0	28,143
01050	30,700				30,700
01060	38,696				38,696
04020	5,165				5,165
04050	8,983				8,983
04090	45,852	34,434	0	34,434	11,418
05030	120,971	40,443	40,443	0	80,528
05040	46,341	7,583	0	7,583	38,758
05050	1,354				1,354
05070	3,009				3,009
05090	8,325	1,204	0	1,204	7,121
06050	153,489	78,097	16,801	61,296	75,392
06051	346,903	293,405	212,328	81,077	53,498
06060	8,231				8,231
06080	11,381	11,381	0	11,381	0
08020	287,677	173,554	172,422	2,788	114,123
08060	858,921	305,532	186,717	209,179	553,389
08070	26,419	6,860	3,299	6,860	19,559
08090	26,990	12,296	11,086	1,660	14,694
09010	33,000	1,062	1,062	0	31,938
10020	13,600	10,880	10,880	0	2,720
10050	139,681	120,364	120,364	0	19,317
10080	16,719	2,253	2,253	0	14,466
12010	32,103	2,122	2,122	0	29,981
12030	23,846	13,291	13,291	0	10,555
12050	22,052	21,016	21,016	0	1,036
13010	23,207	16,258	16,258	0	6,949
13020	405				405
13030	244	244	244	0	0
13040	1,143	259	259	0	884
13050	11,472	2,126	2,126	0	9,346
13060	45,531	1,099	1,099	0	44,432
13070	116,002	21,994	21,212	782	94,008
13080	50,999	2,469	2,469	0	48,530
13090	31,000	2,094	2,094	0	28,906

CRD Numeric	Planted	Treated Base Acres	Soil Treated Base Acres	Foliar Treated Base Acres	Non Treated Base Acres
16070	67,128	2,186	2,186	0	64,942
16080	54,926	5,155	4,791	364	49,771
16090	52,946				52,946
17010	1,710,990	785,864	723,053	62,811	925,126
17020	1,046,005	298,635	298,635	0	747,370
17030	1,025,010	413,595	402,051	11,545	611,415
17040	1,482,998	576,869	576,869	0	906,129
17050	1,526,002	1,194,112	1,194,112	0	331,890
17060	1,461,016	578,679	551,175	80,172	882,337
17070	1,493,004	607,656	586,697	21,239	885,348
17080	599,186	254,277	240,301	13,976	344,909
17090	555,803	113,776	107,804	5,972	442,027
18010	943,999	738,560	738,560	142,408	205,439
18020	791,003	305,422	305,422	0	485,581
18030	583,994	147,091	125,942	23,948	436,903
18040	714,001	385,669	385,669	0	328,332
18050	1,208,995	396,205	371,516	24,689	812,790
18060	443,044	112,696	112,696	0	330,348
18070	797,000	447,838	439,723	8,116	349,162
18080	74,274	21,105	21,105	0	53,169
18090	343,679	17,763	17,763	0	325,916
19010	1,805,002	298,391	298,391	0	1,506,611
19020	1,675,995	222,223	201,472	20,752	1,453,772
19030	1,439,993	452,420	452,100	320	987,573
19040	1,734,002	234,033	219,639	14,394	1,499,969
19050	1,676,001	86,720	62,098	24,622	1,589,281
19060	1,238,998	231,582	217,701	13,881	1,007,416
19070	817,029	103,263	103,263	0	713,766
19080	613,195	93,235	93,235	0	519,960
19090	799,771	231,685	231,685	0	568,086
20010	520,286	205,947	52,107	175,889	314,339
20020	329,087	91,171	91,171	0	237,916
20030	922,629	567,295	52,476	514,819	355,334
20040	356,067	34,488	34,488	0	321,579
20050	100,346				100,346
20060	286,587	41,491	10,280	31,211	245,096
20070	408,997	75,816	75,816	0	333,181
20080	245,222	4,281	4,281	0	240,941
20090	130,775	36,633	36,633	0	94,142
21010	256,998	54,926	32,008	28,284	202,072
21020	605,999	246,798	246,798	0	359,201
21030	242,002	20,838	20,838	797	221,164
21040	37,001	13,238	13,238	0	23,763

CRD Numeric	Planted	Treated Base Acres	Soil Treated Base Acres	Foliar Treated Base Acres	Non Treated Base Acres
21050	101,001	43,725	43,725	7,108	57,276
21060	37,001	1,616	1,616	0	35,385
22010	1,816				1,816
22030	81,802	3,550	3,308	242	78,252
22040	121,344				121,344
22050	59,904	5,263	2,991	2,877	54,641
22060	13,984				13,984
22070	1,151				1,151
23010	321				321
23020	25,412				25,412
23030	267	10,414	10,414	6,521	-10,147
24010	18,682	2,877	2,877	0	15,805
24020	109,943	30,999	30,999	0	78,944
24030	141,353	35,081	35,081	0	106,272
24080	71,884	2,197	2,197	0	69,687
24090	168,138	79,227	71,886	7,341	88,911
25010	21,999	1,925	1,925	0	20,074
26010	39,610				39,610
26020	49,386				49,386
26030	36,792				36,792
26040	73,651	2,321	0	2,321	71,330
26050	227,563	724	724	0	226,839
26060	440,003	35,321	8,620	26,701	404,682
26070	342,997	192,417	189,293	3,124	150,580
26080	673,003	136,123	136,123	0	536,880
26090	317,001	12,838	12,838	0	304,163
27010	134,703	1,942	0	1,942	132,761
27020	79,289				79,289
27030	9,331				9,331
27040	1,276,004	54,269	50,523	3,746	1,221,735
27050	1,387,005	69,225	69,225	0	1,317,780
27060	211,681	39,881	39,881	0	171,800
27070	1,373,005	66,975	66,975	0	1,306,030
27080	1,449,004	33,880	30,328	3,552	1,415,124
27090	979,997	208,649	201,523	7,127	771,348
28010	3,286	2,091	2,091	1,494	1,195
28020	4,722				4,722
28030	97,282	6,049	0	6,049	91,233
28040	118,945				118,945
28050	3,454	259	259	0	3,195
28060	5,783	597	597	0	5,186
28070	86,611				86,611
28080	39,820	39,820	39,820	0	0

CRD Numeric	Planted	Treated Base Acres	Soil Treated Base Acres	Foliar Treated Base Acres	Non Treated Base Acres
28090	40,096				40,096
29010	672,003	172,209	172,209	0	499,794
29020	284,000	164,480	148,445	16,035	119,520
29030	445,995	165,808	165,808	0	280,187
29040	225,434	95,208	88,755	27,963	130,226
29050	394,580	239,525	222,532	41,192	155,055
29060	230,238	103,647	103,647	0	126,591
29070	32,087	5,039	5,039	0	27,048
29080	8,898				8,898
29090	406,763	32,955	32,955	0	373,808
30030	23,263				23,263
30080	13,709				13,709
30090	23,028				23,028
31010	590,588	107,660	102,570	5,090	482,928
31020	483,283	106,178	70,248	35,930	377,105
31030	1,509,002	270,563	251,209	21,560	1,238,439
31050	1,107,003	753,000	721,393	31,608	354,003
31060	1,943,012	633,976	628,575	5,401	1,309,036
31070	632,129	294,402	240,537	53,865	337,727
31080	852,999	496,185	496,185	509	356,814
31090	1,082,001	345,958	345,958	0	736,043
32010	3,000				3,000
33010	15,000				15,000
34020	36,403	1,126	1,126	0	35,277
34050	21,313	9,264	8,260	1,004	12,049
34080	22,284				22,284
35030	133,606	112,550	64,228	48,322	21,056
35090	16,394	4,592	0	4,592	11,802
36020	108,144	1,199	1,199	0	106,945
36030	55,989	11,211	8,963	2,248	44,778
36040	328,494	75,713	75,713	0	252,781
36050	256,998	68,960	68,960	0	188,038
36060	98,870	29,128	29,128	0	69,742
36070	146,507	51,823	51,823	0	94,684
36080	33,660	10,348	10,348	0	23,312
36090	57,142	34,577	34,577	0	22,565
36091	14,199	1,493	1,493	0	12,706
37010	25,568	2,213	2,213	0	23,355
37020	32,430	27,038	27,038	0	5,392
37040	37,000				37,000
37050	96,564	5,113	5,113	0	91,451
37060	27,436	2,763	2,763	0	24,673
37070	154,001	100,040	100,040	948	53,961

CRD Numeric	Planted	Treated Base Acres	Soil Treated Base Acres	Foliar Treated Base Acres	Non Treated Base Acres
37080	176,001	96,540	96,540	0	79,461
37090	161,000	40,415	40,415	0	120,585
38010	2,240				2,240
38020	22,893				22,893
38030	71,865				71,865
38040	5,208				5,208
38050	83,792				83,792
38060	145,000	2,017	0	2,017	142,983
38070	44,260				44,260
38080	76,740				76,740
38090	348,006	2,481	2,481	0	345,525
39010	697,004	69,185	69,065	120	627,819
39020	478,999	68,168	53,278	14,890	410,831
39030	208,683	52,027	52,027	0	156,656
39040	697,000	132,273	132,273	0	564,727
39050	660,000	112,302	102,950	9,353	547,698
39060	151,314	68,099	68,099	0	83,215
39070	266,340	102,430	100,620	1,810	163,910
39080	108,257	22,596	22,596	0	85,661
39090	132,402	23,481	23,481	0	108,921
40010	153,277	30,046	8,456	25,188	123,231
40020	5,437				5,437
40030	14,992				14,992
40040	301				301
40050	18,160				18,160
40060	2,988				2,988
40070	19,344				19,344
40080	47,264				47,264
40090	8,237				8,237
41010	5,997	2,679	2,679	0	3,318
41080	54,003	39,310	39,310	0	14,693
42010	145,235	19,454	18,410	2,800	125,781
42020	66,765	23,979	22,574	1,405	42,786
42030	52,215	25,696	25,696	0	26,519
42040	108,257	26,123	26,123	0	82,134
42050	299,996	107,700	107,700	0	192,296
42060	77,787	35,874	35,874	0	41,913
42070	57,742	14,613	14,613	0	43,129
42080	287,001	85,994	85,994	0	201,007
42090	405,000	251,399	248,596	2,803	153,601
44010	2,000				2,000
45010	10,229				10,229
45030	80,610	62,042	62,042	0	18,568

CRD Numeric	Planted	Treated Base Acres	Soil Treated Base Acres	Foliar Treated Base Acres	Non Treated Base Acres
45040	8,617				8,617
45050	80,322	10,020	10,020	0	70,302
45080	100,221	4,880	4,880	0	95,341
46010	62,090	42,785	42,785	0	19,305
46020	589,000				589,000
46030	554,998	3,496	0	3,496	551,502
46040	5,785				5,785
46050	561,995				561,995
46060	877,994	86,155	86,155	0	791,839
46070	43,786				43,786
46080	188,339				188,339
46090	915,998	37,148	37,148	0	878,850
47010	209,194	24,142	20,135	4,007	185,052
47020	170,806	7,012	7,012	0	163,794
47030	74,000	22,152	22,152	0	51,848
47040	72,000	20,433	15,801	4,631	51,567
47050	60,000	7,399	7,399	0	52,601
47060	44,002	18,133	15,891	2,242	25,869
48011	469,812	207,087	17,895	194,491	262,725
48012	126,373	57,116	0	57,116	69,257
48021	1,957				1,957
48040	405,026	160,434	160,434	0	244,592
48051	20,945	14,301	14,301	0	6,644
48052	148,432	148,432	148,432	0	0
48070	11,431	7,647	7,647	0	3,784
48081	223,875	198,273	197,776	497	25,602
48082	3,731	281	281	0	3,450
48090	136,539	115,662	115,662	0	20,877
48096	18,226	6,182	6,182	0	12,044
48097	33,670	3,288	0	3,288	30,382
49010	22,056	8,347	6,997	1,350	13,709
49050	31,284	9,148	9,148	0	22,136
49060	6,661				6,661
50010	90,002	6,739	5,638	1,201	83,263
51020	123,132	41,110	41,110	0	82,022
51040	22,210	11,677	11,677	0	10,533
51050	31,930	18,875	18,875	0	13,055
51060	158,936	30,416	22,726	7,690	128,520
51070	24,789	8,953	8,953	0	15,836
51080	13,790	4,325	4,325	0	9,465
51090	55,213	5,992	5,992	0	49,221
53010	50,293				50,293
53020	8,801				8,801



CRD Numeric	Planted	Treated Base Acres	Soil Treated Base Acres	Foliar Treated Base Acres	Non Treated Base Acres
53050	35,326	416	0	416	34,910
53090	20,581				20,581
54020	8,181	862	862	0	7,319
54040	21,439	17,962	17,962	0	3,477
54060	25,379	12,507	12,507	3,250	12,872
55010	313,822	39,424	38,795	629	274,398
55020	216,400				216,400
55030	129,776				129,776
55040	534,002	131,838	115,623	16,214	402,164
55050	271,999	28,785	28,785	0	243,214
55060	513,001	65,281	65,281	0	447,720
55070	496,001	159,547	149,901	9,646	336,454
55080	700,001	224,248	224,248	0	475,753
55090	225,002	23,648	23,648	0	201,354
56010	36,158	5,348	5,348	0	30,810
56050	48,843	3,679	3,679	1,982	45,164
Total	76,009,055	21,168,694	19,184,522	2,387,397	54,840,361

**Note:**

Source: 2001 Doane Agro-Trak Study -- Doane Marketing Research, Inc., St. Louis, Missouri.

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**[00053]** Data is also available to indicate those crop reporting districts in which no insecticide use on corn was reported anywhere in the CRD. For the year 2001, for example, Table 2 shows that CRD's having a total of over 3 million acres reported no insecticide use on corn.

10

**Table 2:** Corn acreage that is grown with no insecticide applications in 2001 by U.S. crop reporting district.

CRD Numeric	Planted Acres
01010	36638
01020	46741
01030	17041
01050	30700
01060	38696

CRD Numeric	Planted Acres
04020	5165
04050	8983
05050	1354
05070	3009
06060	8231

CRD Numeric	Planted Acres
13020	405
16090	52946
20050	100346
22010	1816
22040	121344
22060	13984
22070	1151
23010	321
23030	267
26010	39610
26020	49386
26030	36792
27020	79289
27030	9331
28020	4722
28040	118945
28070	86611
28090	40096
29080	8898
30030	23263
30080	13709
30090	23028
32010	3000
33010	15000
34080	22284
37040	37000
38010	2240
38020	22893
38030	71865
38040	5208
38050	83792
38070	44260
38080	76740
40020	5437
40030	14992
40040	301
40050	18160
40060	2988
40070	19344
40080	47264
40090	8237
44010	2000

CRD Numeric	Planted Acres
45010	10229
45040	8617
46020	589000
46040	5785
46050	561995
46070	43786
46080	188339
48021	1957
49060	6661
53010	50293
53020	8801
53090	20581
55020	216400
55030	129776
Total	3,368,043

Note:

Source: 2001 Doane AgroTrak Study -- Doane Marketing Research, Inc.,  
St. Louis, Missouri.

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[00054] In some CRD's, very little insecticide is used on corn. This is  
believed to indicate that while insecticide may be used in one, or a few,  
locations within a CRD, other locations within a CRD have no insecticide  
use at all. For example, it may be that while insecticides are used on corn  
10 in one county within a CRD, other counties within the same CRD would  
have no insecticide use on corn. Accordingly, it is believed that the level  
of insect infestation in those counties is below that where insecticide use is  
indicated.

[00055] Data on insecticide use for crops other than corn is also  
15 available. Table 3, for example, shows insecticide use on cotton acreage  
in the U.S. in 2001 by CRD. It can be seen that over one-half of the 16.1  
million acres planted to cotton received no insecticide treatment. These  
locations, therefore, would be presumed to have levels of insect infestation  
that were below that at which insecticide treatment would be indicated.

20 Table 3: Cotton acreage that is treated and untreated with  
insecticide in the U.S. in 2001 by crop reporting district.

CRD Numeric	Planted	Treated Base Acres	Soil Treated Base Acres	Foliar Treated Base Acres	Non Treated Base Acres
01010	224,001	134,050	118,985	25,580	89,951
01020	38,403	31,735	7,987	23,748	6,668
01030	31,597				31,597
01040	62,001	42,975	10,565	37,231	19,026
01050	104,006	84,789	73,099	56,933	19,217
01060	149,998	62,011	26,082	42,084	87,987
04050	194,001	131,763	115,677	34,987	62,238
04070	42,675	19,287	7,826	11,461	23,388
04090	49,326	20,057	20,057	1,690	29,269
05030	559,973	459,015	359,296	254,736	100,958
05050	16,277				16,277

CRD Numeric	Planted	Treated Base Acres	Soil Treated Base Acres	Foliar Treated Base Acres	Non Treated Base Acres
05060	274,725	233,754	215,278	178,615	40,971
05070	16,747	16,301	16,301	7,377	446
05090	302,249	257,163	222,557	148,460	45,086
06040	15,187	9,051	9,051	0	6,136
06050	9,621	9,621	6,591	9,621	0
06051	781,186	686,973	583,146	338,150	94,213
06080	19,002	14,457	14,457	11,467	4,545
12010	119,913	57,852	36,184	34,611	62,061
12030	5,088	5,088	5,088	5,088	0
13030	2,692	2,692	2,692	2,692	0
13040	11,428	2,244	2,244	0	9,184
13050	150,878	63,620	55,109	27,358	87,258
13060	197,001	103,669	82,338	35,975	93,332
13070	448,002	223,152	166,067	121,489	224,850
13080	576,007	326,073	251,726	174,616	249,934
13090	114,001	83,146	64,927	66,139	30,855
20060	32,193	22,771	19,041	7,459	9,422
20090	11,807				11,807
22010	72,173	70,476	69,028	59,652	1,697
22020	9,827	8,828	8,828	2,876	999
22030	610,005	495,803	348,946	276,107	114,202
22050	217,772	207,064	203,484	28,891	10,708
22060	227	53,474			-53,247
28010	434,001		400,361	66,967	434,001
28020	138,726		88,401	31,703	138,726
28030	46,270		24,404	18,768	46,270
28040	745,992		681,705	280,740	745,992
28050	201,999		126,604	110,239	201,999
28060	97,411		41,721	46,020	97,411
28070	29,569	20,116	20,116	3,545	9,453
28090	6,022	5,751	5,751	0	271
29040	7,074	7,074	0	7,074	0
29070	6,082	1,839	0	1,839	4,243
29090	386,848	212,146	120,555	99,647	174,702
35030	22,987	1,379	276	1,379	21,608
35070	15,013	1,021	1,021	1,021	13,992
35090	44,002	18,691	17,706	1,773	25,311
37050	9,129	9,129	3,485	8,839	0
37060	39,869	19,251	0	19,251	20,618
37070	449,006	342,415	310,303	150,380	106,591
37080	316,004	172,135	165,404	98,612	143,869
37090	246,001	128,661	102,906	87,010	117,340

CRD Numeric	Planted	Treated Base Acres	Soil Treated Base Acres	Foliar Treated Base Acres	Non Treated Base Acres
40010	984				984
40020	52,039	1,326	1,326	0	50,713
40030	192,993	95,247	93,644	10,215	97,746
40040	2,531	1,205	1,205	0	1,326
40060	1,446				1,446
45010	1,262				1,262
45020	769	769	0	769	0
45030	142,003	100,010	94,434	30,444	41,993
45040	13,463	9,076	9,076	5,169	4,387
45050	100,998	73,675	69,726	18,080	27,323
45080	41,506	37,499	37,499	22,273	4,007
47010	207,001	83,399	77,836	24,830	123,602
47020	373,391	284,890	248,742	117,732	88,501
47030	13,284				13,284
47040	10,325				10,325
47060	6,003	6,003	6,003	6,003	0
48011	889,009	180,233	83,983	111,518	708,776
48012	2,872,020	434,271	154,605	326,335	2,437,749
48021	398,000	37,833	18,984	18,848	360,167
48022	678,000	34,887	34,887	0	643,113
48030	3,228				3,228
48040	175,713	160,882	159,683	68,330	14,831
48052	14,058	14,058	14,058	8,461	0
48060	31,084				31,084
48070	266,914	18,415	18,415	11,112	248,499
48081	42,452	42,452	26,957	40,651	0
48082	297,998	290,816	241,938	48,878	7,182
48090	293,549	270,302	257,790	90,542	23,247
48096	21,951	21,951	21,951	16,978	0
48097	233,052	186,088	186,088	21,027	46,964
51060	11,167	6,890	6,890	2,284	4,277
51090	93,835	62,536	54,292	31,510	31,299
Total	16,194,022	7,167,490	7,026,416	4,042,561	9,026,532

Note:

Source: 2001 Doane AgroTrak Study -- Doane Marketing Research, Inc.,  
St. Louis, Missouri.

5

[00056] Another method of determining whether the level of infestation  
by the insect that is a pest for the agronomic plant indicates treatment with

an insecticide involves comparing a level of infestation by the insect at the location with a level of infestation by the insect at which treatment with an insecticide would be indicated. By way of example, this can be accomplished by determining the level of infestation by the insect at the location, and determining a level of infestation by the insect at which treatment with an insecticide would be indicated. When these two levels of infestation have been determined, they are compared to see which is higher. In other words, to determine whether or not to treat the seed with an insecticide in order to reduce or avoid expected insect damage. Then, if the level of infestation of the location by the insect is lower than the level of infestation at which treatment is indicated, to treat the seed with a neonicotinoid insecticide.

[00057] In the present method, the step of "determining the level of infestation by the insect at the location" is meant to include the acquisition of knowledge about the level of infestation in any manner and from any source, including, without limitation, direct tests, written or oral reports, discussions with agricultural extension personnel, county agents, radio reports, agricultural bulletins, anecdotal data derived from discussions with neighboring farmers or other persons knowledgeable about the level of insect pest infestation of the location, such as agricultural equipment and materials suppliers, producers, wholesalers, retailers, and consultants, as well as from historical data, recommendations by seed or pesticide manufacturers and suppliers, and the like.

[00058] In preferred embodiments, the level of insect pest infestation at the location is determined by measurement of the level of infestation of insects against which neonicotinoid insecticides have insecticidal activity, and which are pests for the agronomic plants that one expects to grow, where the measurement is carried out at or near the location one expects to grow the plants. Examples of how such measurements can be made include the visual inspection of plants, setting out non-specific lures and traps, and by setting out genus or species-specific lures and traps. Such

testing and measurement techniques are well known in the art of insect pest management.

[00059] The level of infestation by the insect at which treatment with an insecticide would be indicated can be determined on any basis that is of interest to the practitioner. By way of example, one common basis is an economic determination -- e.g. cost vs. value. One can compare the cost of applying a neonicotinoid insecticide with the expected value of the added yield due to reduced insect damage. If the cost is less than the expected added value, then treatment with an insecticide would be indicated. On the other hand, if the cost is more than the expected value of the yield added due to reduced insect damage, then treatment with an insecticide would not be indicated. Of course, if the level of insect infestation is zero, or close to zero, then treatment with an insecticide would not be indicated in any case.

[00060] An example of another basis for this determination is an objective standard, such as the expected level of corn rootworm (CRW) damage. A test for corn rootworm damage can be carried out by the Iowa Root Rating Method, which is described below and is a test that assesses damage on a 1 - 6 scale (from least damage to worst damage). If historical data show CRW damage level of less than about 3, then treatment with an insecticide active against CRW would not be indicated, whereas CRW damage of above about 3 would indicate the need for insecticide treatment. In preferred embodiments, a CRW damage level of below 2.6 would indicate that no insecticide for corn rootworm protection was needed, more preferred would be a CRW damage level of below 2.0.

[00061] After the level of infestation by the insect at the location and a level of infestation by the insect at which treatment with an insecticide would be indicated are determined, the two are compared. In one embodiment of the present invention, if the level of infestation at the location is below the level at which treatment with an insecticide would be indicated, the seed is treated with a neonicotinoid compound.

[00062] In another embodiment, if the level of infestation at the location is below the level at which treatment with an insecticide would be indicated, the method includes the step of recommending the purchase of a seed that has been treated with a neonicotinoid compound for planting in the location. Included in the meaning of the terms "recommending for purchase" is the act of advertising seed that have been treated with a neonicotinoid compound, or advertising the desirability of treating seed with a neonicotinoid compound, for planting in the location. The action of recommending can be carried out orally, or in writing. It can be published, or non-published. The recommendation can consist only of a suggestion that treatment of seed with a neonicotinoid compound for planting in the location can result in beneficial results.

[00063] In another embodiment, if the level of infestation at the location is below the level at which treatment with an insecticide would be indicated, the method includes the step of selling a seed that has been treated with a neonicotinoid compound for planting in the location. Included within the term "selling" are commercial and non-commercial sales of seed that has been treated with a neonicotinoid compound, as long as the seed are planted, or are meant for planting, in a location having an insect infestation level below that at which insecticide treatment is indicated. Also included within the term "selling", are exchanges, barter, and other forms of trade.

[00064] In another embodiment, if the level of infestation at the location is below the level at which treatment with an insecticide would be indicated, the method involves planting in the location a seed that has been treated with a neonicotinoid compound. The act of planting includes planting a seed directly in the ground as well as the transplantation of a plant that is grown from a seed. Planting can be by hand, by machine, commercial, or non-commercial, without limitation.

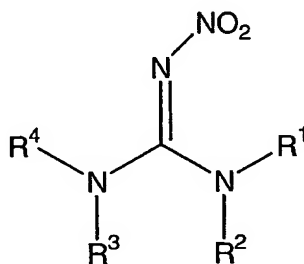
[00065] The "neonicotinoid compound" of the present invention is any neonicotinoid compound that provides the yield and/or vigor-enhancing properties that are the advantages of the present invention when the



compound is used as a seed treatment according to the method described herein. In preferred embodiments, the neonicotinoid compound is one having insecticidal properties.

[00066] Neonicotinoid compounds that are useful in the present invention include those listed in *The Pesticide Manual*, 12 ed., namely, acetamiprid, imidacloprid, thiamethoxam, clothianidin (TI-435), dinotefuran and nitenpyram. Useful neonicotinoid compounds can include nicotinoid insecticides of the type of nitroguanidine insecticides, nitromethylene insecticides, and pyridylmethylamine insecticides, as listed in the *Compendium of insecticide common names*, at [http://www.hclrss.demon.co.uk/class\\_insecticides.html](http://www.hclrss.demon.co.uk/class_insecticides.html) (02/19/2002). Useful neonicotinoid compounds can include the nitroguanidine compounds described at <http://www.nigu.de/pdf/nq-chemistry21.pdf> (07/08/02). Neonicotinoid compounds such as flonicamid, nithiazine and thiacloprid are also included.

[00067] When the neonicotinoid compound is a nicotinoid insecticide of the nitroguanidino type, compounds that are useful in the present invention include a compound having the formula:



where:

$\text{R}^1$  is hydrogen, or  $\text{C}_1$  -  $\text{C}_4$  alkyl;

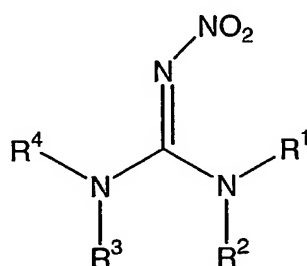
$\text{R}^2$  is hydrogen,  $\text{C}_1$  -  $\text{C}_4$  alkyl,  $\text{C}_1$  -  $\text{C}_4$  alkenyl,  $\text{C}_1$  -  $\text{C}_4$  alkynyl, hydroxyl, amino, aryl, thio, alkylaryl, arylalkyl, or  $\text{C}_4$  -  $\text{C}_6$  heterocyclic;

$\text{R}^3$  is hydrogen,  $\text{C}_1$  -  $\text{C}_4$  alkyl,  $\text{C}_1$  -  $\text{C}_4$  alkenyl,  $\text{C}_1$  -  $\text{C}_4$  alkynyl, hydroxyl, amino, aryl, thio, alkylaryl, arylalkyl, or 4 - 6-member

heterocyclic; and  $R^2$  and  $R^3$  can join to form a 4 - 6 member heterocyclic, that may optionally be substituted or unsubstituted; and

$R^4$  is hydrogen,  $C_1 - C_4$  alkyl,  $C_1 - C_4$  alkenyl,  $C_1 - C_4$  alkynyl, hydroxyl, amino, aryl, thio, alkylaryl, arylalkyl,  $C_4 - C_6$  heterocyclic, halothiazoylalkyl, or furylalkyl.

**[00068]** When the neonicotinoid compound is a nicotinoid insecticide of the nitroguanidino type, compounds that are preferred for use in the present invention include a compound having the formula:



where:

$R^1$  is hydrogen, or methyl;

$R^2$  is hydrogen, or methyl;

$R^3$  is hydrogen, or methyl, or of a form that can join with  $R^2$  to form an oxadiazine ring or a 2,3-diazol ring; and

$R^4$ , if present, is chlorothiazoylmethyl, or furylmethyl.

**[00069]** It is believed that the present method of increasing yield and/or vigor can be used with the seeds of non-transgenic plants, or with the seeds of plants that have at least one transgenic event.

**[00070]** In an embodiment of the present method, the yield and/or vigor of a transgenic agronomic plant can be increased by treating a seed of the transgenic agronomic plant with a neonicotinoid compound, as described above, where the seed comprises a foreign polynucleotide sequence capable of encoding and expressing an insecticidal protein at insecticidally useful levels. This foreign polynucleotide sequence, along with all other genes necessary for the expression of the active protein at useful levels, can be referred to herein as a "transgenic event". A transgenic event in a

seed, or plant, therefore, includes the ability to express a protein. When it is said that a "transgenic event has activity against a pest", it is to be understood that it is the protein that is encoded by the gene that actually has such activity when the protein is expressed and brought into contact with the pest.

[00071] Examples of transgenic events that are useful in the present invention, seeds and plants that comprise such events, as well as examples of methods for their use, can be found in U.S. Patent Nos. 6,329,504, 6,326,351, 6,326,169, 6,316,407, 6,313,378; 6,288,312; 6,284,949; 6,281,016; 6,255,560, 6,248,536, 6,242,241; 6,221,649; 6,218,145; 6,215,048; 6,211,430; 6,197,747; 6,177,615; 6,174,724, 6,156,573; 6,153,814; 6,140,075; 6,121,436, 6,114,610; 6,110,464; 6,093,695; 6,063,756; 6,063,597; 6,060,594, 6,023,013; 6,018,100; 5,962,264; 5,959,091; 5,942,658, 5,880,275; 5,877,012, 5,869,720; 5,859,347; 5,763,241; 5,759,538; 5,679,343; 5,616,319; 5,495,071; 5,424,412; 5,378,619; 5,349,124; 5,250,515; and 5,229,112, among others, and in WO 01/49834, WO 98/13498, WO 00/66742, and WO 99/31248.

[00072] WO 99/31248 and U.S. Patent Nos. 6,326,351, 6,281,016, 6,063,597, 6,060,594 and 6,023,013 describe methods for genetically engineering *B. thuringiensis*  $\delta$ -endotoxin genes so that modified  $\delta$ -endotoxins can be expressed. The modified  $\delta$ -endotoxins differ from the wild-type proteins by having specific amino acid substitutions, additions or deletions as compared with the proteins produced by the wild-type organism. Such modified  $\delta$ -endotoxins are identified herein by the use of an asterisk (\*), or by reference to a specific protein by its identifying number.

[00073] Preferred types of genetically modified Cry\* insect toxins are described in U.S. Patent No. 6,326,169, and include the proteins encoded by polynucleotide sequences that are contained in the *B. thuringiensis* strains deposited as NRRL B-21579, NRRL B-21580, NRRL B-21581, NRRL B-21635, and NRRL B-21636.

[00074] Preferred types of genetically modified Cry\* insect toxins are described in U.S. Patent No. 6,281,016, and include those produced by *B. thuringiensis* strains EG11060, EG 11062, EG11063, EG11065, EG11067, EG11071, EG11073, EG11074, EG11087, EG11088, EG11090, EG11091, EG11092, EG11735, EG11751 and EG11768.

[00075] Preferred types of genetically modified Cry\* insect toxins are described in U.S. Patent No. 6,023,013, and include the proteins encoded by polynucleotide sequences that are contained in the *B. thuringiensis* strains deposited as NRRL B-21744, NRRL B-21745, NRRL B-21746, NRRL B-21747, NRRL B-21748, NRRL B-21749, NRRL B-21750, NRRL B-21751, NRRL B-21752, NRRL B-21753, NRRL B-21754, NRRL B-21755, NRRL B-21756, NRRL B-21757, NRRL B-21758, NRRL B-21759, NRRL B-21760, NRRL B-21761, NRRL B-21762, NRRL B-21763, NRRL B-21764, NRRL B-21765, NRRL B-21766, NRRL B-21767, NRRL B-21768, NRRL B-21769, NRRL B-21770, NRRL B-21771, NRRL B-21772, NRRL B-21773, NRRL B-21774, NRRL B-21775, NRRL B-21776, NRRL B-21777, NRRL B-21778, and NRRL B-21779.

[00076] Preferred types of genetically modified Cry\* insect toxins are described in U.S. Patent No. 6,063,597, and include , without limitation: Cry3Bb.11230, Cry3Bb.11231, Cry3Bb.11232, Cry3Bb.11233, Cry3Bb.11234, Cry3Bb.11235, Cry3Bb.11236, Cry3Bb.11237, Cry3Bb.11238, Cry3Bb.11239, Cry3Bb.11241, Cry3Bb.11242, and Cry3Bb.11098.

[00077] Some of the modified  $\delta$ -endotoxins that were described in WO 99/31248 and in U.S. Patent No. 6,063,597 were found to have enhanced activity against coleopteran insects, and in particular against *Diabrotica spp.*, including corn rootworm. As used herein, the terms "enhanced activity" refer to the increased insecticidal activity of a modified toxin as compared with the activity of the same toxin without the amino acid modifications when both are tested under the same conditions. In particular, it was found that Cry3\*  $\delta$ -endotoxins had enhanced activity against corn rootworm, and are therefore preferred for use in the present

invention when corn seed is being treated. More preferred are Cry3B\*  $\delta$ -endotoxins, and even more preferred are Cry3Bb\*  $\delta$ -endotoxins. Even more preferred transgenic events are those that comprise the ability to express the modified  $\delta$ -endotoxins that are listed below in Table 4.

5 Also shown in the table are strains of transgenic *B. thuringiensis* that include genes for expression of the respective novel endotoxins, and the date and accession number of their deposit with the Agricultural Research Service Culture Collection (NRRL) at 1815 N. University Street, Peoria, IL 61604.

10 Table 4: *B. thuringiensis* strains expressing modified toxic proteins.

STRAIN	DEPOSIT DATE	PROTEIN	ACCESSION NUMBER (NRRL NUMBER)
EG11230	5/27/97	Cry3Bb.11230	B-21768
EG11231	5/27/97	Cry3Bb.11231	B-21769
EG11232	5/27/97	Cry3Bb.11232	B-21770
EG11233	5/27/97	Cry3Bb.11233	B-21771
EG11234	5/27/97	Cry3Bb.11234	B-21772
EG11235	5/27/97	Cry3Bb.11235	B-21773
EG11236	5/27/97	Cry3Bb.11236	B-21774
EG11237	5/27/97	Cry3Bb.11237	B-21775
EG11238	5/27/97	Cry3Bb.11238	B-21776
EG11239	5/27/97	Cry3Bb.11239	B-21777
EG11241	5/27/97	Cry3Bb.11241	B-21778
EG11242	5/27/97	Cry3Bb.11242	B-21779
EG11098	11/28/97	Cry3Bb.11098	B-21903

15 **[00078]** The present invention also includes the treatment of seeds having more than one transgenic event. Such combinations are referred to as "stacked" transgenic events. These stacked transgenic events can be events that are directed at the same target pest, or they can be directed at different target pests. In one preferred method, a seed having the ability to

express a Cry 3 protein also has the ability to express at least one other insecticidal protein that is different from a Cry 3 protein.

[00079] The present invention also includes the treatment of seeds having one or more transgenic event which encodes for the production of binary insecticidal proteins including, but not limited to, CryET33 and CryET34, CryET80 and CryET76, t1C100 and t1C101, and PS149B1.

[00080] The present invention also includes the treatment of seeds having Herculex® I transgenic events (available from Dow Agrosciences, Mycogen Seeds, and Pioneer Hi-Bred International).

[00081] In an embodiment of the present invention where the subject method includes treatment of the seed and/or the foliage of a plant with a herbicide or with a pesticide other than a neonicotinoid, it is preferred that the plant be a transgenic plant having a transgenic event that confers resistance to the particular herbicide or other pesticide that is employed.

[00082] When a herbicide such as glyphosate is included in the treatment, it is preferred that the transgenic plant or plant propagation material be one having a transgenic event that provides glyphosate resistance. Some examples of such preferred transgenic plants having transgenic events that confer glyphosate resistance are described in U.S.

Patent Nos. 6,248,876, 6,225,114, 6,107,549, 5,866,775, 5,804,425, 5,776,760, 5,633,435, 5,627,061, 5,463,175, 5,312,910, 5,310,667, 5,188,642, 5,145,783, 4,971,908 and 4,940,835. When the transgenic plant is a transgenic soybean plant, such plants having the characteristics of "Roundup-Ready" transgenic soybeans (available from Monsanto Company, St. Louis, MO) are preferred.

[00083] The present invention is also useful for application to the seeds of plants which have been improved by a program of selective breeding based on quantitative trait loci (QTL) information. Further information about the use of such breeding programs can be found in U.S. Patent No. 5,476,524, and in Edwards, M. D. *et al.*, *Genetics*, 116:113 - 125 (1987); Edwards, M. D. *et al.*, *Theor. Appl. Genet.*, 83:765 - 774 (1992); Paterson, A. H. *et al.*, *Nature*, 335:721 - 726 (1988); and Lander, E. S. *et al.*,

*Mapping Medelian Factors Underlying Quantitative Traits Using RFLP Linkage Maps*, Genetics Society of America, pp. 185 - 199 (1989).

[00084] In one embodiment, the present method is particularly useful when used as a part of a conventional yield-enhancing breeding program for a crop. This is particularly useful when the breeding program is for a transgenic crop. As mentioned above, transgenic events are initially transferred into plant strains that are stable, vigorous and have good records as parents in hybridizing trials, but are not usually themselves high-yielding hybrids. The transgenic strains are then hybridized with other parents in conventional breeding programs, to arrive at high-yielding hybrids that also contain the desired transgenic event(s). As mentioned above, one disadvantage that is a common feature of the commercial high-yielding hybrids -- and particularly for transgenic hybrids, is that they are not as vigorous as the parent. In some cases, for example, transgenic hybrid corn plants have significantly smaller root systems than their parents. This can cause higher sensitivity to root-damaging pests, as well as to lodging.

[00085] In one embodiment, the present method is applied to the seeds that are used in a breeding program. In particular, the method can be applied to a breeding program in which at least one parent is a transgenic plant. Also, the present method is useful, as explained above, as a treatment for high-yielding transgenic seeds that are the product of the breeding trial.

[00086] In a method of breeding a hybrid plant from two parent plants, the method comprises treating the seeds of one or both of the parent plants with a neonicotinoid compound prior to planting the seeds; pollinating the female parent with pollen of the male parent; and gathering the seed produced by the female parent plant.

[00087] In a preferred embodiment, one or both of the parent plants contain a foreign gene that encodes for the production of a pesticidal protein. It is further preferred that the pesticidal protein comprises an insect toxin.

[00088] Plants which are suitable for the practice of the present invention include any gymnosperm and angiosperm, including dicotyledons and monocotyledons. Preferred plants are those which are agronomically important. Examples of agronomically important plants include, for example, plants that are edible in part or in whole by a human or an animal. Edible plants that may be useful in the present invention are not particularly limited and may be gymnosperms, angiosperms, including monocotyledons and dicotyledons. Such plants include cereals (wheat, barley, rye, oats, rice, sorghum, related crops, etc.), beet, pear-like fruits, stone fruits, and soft fruits (apple, pear, plum, peach, Japanese apricot, prune, almond, cherry, strawberry, raspberry, and black berry, etc.), legumes (kidney bean, lentil, pea, soybean), oil plants (rape, mustard, poppy, olive, sunflower, coconut, castor-oil plant, cocoa bean, peanut, etc.), Cucurbitaceae (pumpkin, cucumber, melon, etc.), citrus (orange, lemon, grape fruit, mandarin, Watson pomelo (citrus natsudaidai), etc.), vegetables (lettuce, cabbage, celery cabbage, Chinese radish, carrot, onion, tomato, potato, green pepper, etc.), camphor trees (avocado, cinnamon, camphor, etc.), corn, tobacco, nuts, coffee, sugar cane, tea, grapevine, hop and banana.

[00089] Edible plants that are particularly useful include rice, wheat, barley, rye, corn, potato, carrot, sweet potato, sugar beet, bean, pea, chicory, lettuce, cabbage, cauliflower, broccoli, turnip, radish, spinach, asparagus, onion, garlic, eggplant, pepper, celery, canot, squash, pumpkin, zucchini, cucumber, apple, pear, quince, melon, plum, cherry, peach, nectarine, apricot, strawberry, grape, raspberry, blackberry, pineapple, avocado, papaya, mango, banana, soybean, tomato, sorghum and raspberries, banana and other such edible varieties.

[00090] The present invention can also be useful for increasing the yield and/or vigor of fiber producing plants including cotton, flax, hemp, jute, ramie, sisal; lumber producing trees including hardwoods and softwoods, such as, pine, oak, redwood, poplar, gum, ash, fir, birch, hemlock, larch, mahogany, ebony, and the like, as well as ornamental shrubs and trees.



[00091] In the method of the present invention, the neonicotinoid compound is applied to a seed. Although it is believed that the present method can be applied to a seed in any physiological state, it is preferred that the seed be in a sufficiently durable state that it incurs no damage during the treatment process. Typically, the seed would be a seed that had been harvested from the field; removed from the plant; and separated from any cob, stalk, outer husk, and surrounding pulp or other non-seed plant material. The seed would preferably also be biologically stable to the extent that the treatment would cause no biological damage to the seed.

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10 In one embodiment, for example, the treatment can be applied to seed corn that has been harvested, cleaned and dried to a moisture content below about 15% by weight.

[00092] In an alternative embodiment, the seed can be one that has been dried and then primed with water and/or another material and then re-dried before or during the treatment with the neonicotinoid compound. Within the limitations just described, it is believed that the treatment can be applied to the seed at any time between harvest of the seed and sowing of the seed. As used herein, the term "unsown seed" is meant to include seed at any period between the harvest of the seed and the sowing of the seed in the ground for the purpose of germination and growth of the plant.

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[00093] In preferred embodiments, the neonicotinoid compound is applied directly to the seed, rather than to the soil in which the seed is, or is to be, planted. In other embodiments, the neonicotinoid compound can be applied to the soil -- for example, by deposition in bands, "T"-bands, or in-furrow, at the same time as the seed is sowed -- as well as directly to the seed. In other embodiments, the neonicotinoid compound can be applied to the seed indirectly, such as by applying the compound to the soil in which the seed is sown.

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[00094] The neonicotinoid compound can be applied "neat", that is, without any diluting or additional components present. However, the compound is typically applied to the seeds in the form of a formulation. This formulation may contain one or more other desirable components

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including but not limited to liquid diluents, binders to serve as a matrix for the neonicotinoid compound, fillers for protecting the seeds during stress conditions, and plasticizers to improve flexibility, adhesion and/or spreadability of the coating. In addition, for oily formulations containing  
5 little or no filler, it may be desirable to add to the formulation drying agents such as calcium carbonate, kaolin or bentonite clay, perlite, diatomaceous earth or any other adsorbent material. Use of such components in seed treatments is known in the art. See, e.g., U.S. Patent No. 5,876,739. The skilled artisan can readily select desirable components to use in the  
10 neonicotinoid compound formulation depending on the seed type to be treated and the particular neonicotinoid compound that is selected. In addition, readily available commercial formulations of known insecticides and other pesticides may be used, as demonstrated in the examples below.

15 **[00095]** The seeds may also be treated with one or more of the following ingredients: pesticides other than neonicotinoid compounds, including compounds which act only below the ground; fungicides, such as captan, thiram, metalaxyl, mefenoxam (resolved isomer of metalaxyl), fludioxonil, oxadixyl, azoxystrobin, ipconazole, and isomers of each of  
20 those materials, and the like; herbicides, including compounds selected from carbamates, thiocarbamates, acetamides, triazines, dinitroanilines, glycerol ethers, pyridazinones, uracils, phenoxys, ureas, and benzoic acids; herbicidal safeners such as benzoxazine, benzhydryl derivatives, N,N-diallyl dichloroacetamide, various dihaloacyl, oxazolidinyl and  
25 thiazolidinyl compounds, ethanone, naphthalic anhydride compounds, and oxime derivatives; fertilizers; and biocontrol agents such as naturally-occurring or recombinant bacteria and fungi from the genera *Rhizobium*, *Bacillus*, *Pseudomonas*, *Serratia*, *Trichoderma*, *Glomus*, *Gliocladium* and mycorrhizal fungi. These ingredients may be added as a separate layer  
30 on the seed or alternatively may be added as part of the treating composition.

[00096] When the seed is treated with pesticides other than neonicotinoid compounds, such pesticides can include fungicides and herbicides; herbicidal safeners; fertilizers and/or biocontrol agents. These ingredients may be added as a separate layer or alternatively may be added in the pesticidal coating layer.

[00097] When the seed is treated with other pesticides, such pesticides can be selected from acaricides, bactericides, fungicides, nematocides and molluscicides.

[00098] When the seed is treated with a fungicide, it is preferably selected from a group consisting of tebuconazole, tetraconazole, simeconazole, difenoconazole, fluquinconazole, fludioxonil, captan, metalaxyl, carboxin, azoxystrobin, ipconazole, and thiram.

[00099] When the seed is treated with a herbicide, it can be selected from the following useful herbicides:

growth regulators, including

phenoxy acetic acids, such as, 2,4-D and MCPA,

phenoxy propionic acids, such as, dichlorprop and mecoprop,

phenoxy butyric acids, such as, 2,4-DB and MCPB,

benzoic acids, such as, dicamba,

picolinic acid and related compounds, such as, picloram, triclopyr,

clopyralid and quinclorac;

inhibitors of auxin transport, including

naptalam,

semicarbones, such as, diflufenzopyr-sodium,

s-triazines, such as, atrazine, simazine, cyanazine, prometon,

ametryn and prometryn,

other triazines, such as, hexazinone and metribuzin,

substituted ureas, such as, diuron, fluometuron, linuron and

tebuthiuron,

uracils, such as, bromacil and terbacil,

benzothiadiazoles, such as, bentazon,

benzonitroles, such as, bromoxymil,

- phenylcarbamates, such as, desmedipham and phenmedipham,  
pyridazinones, such as, pyrazon,  
phenypyridazines, such as, pyridate, and  
others, such as, propanil;
- 5 pigment inhibitors, including  
amitrole, clomazone and fluridone,  
pyridazinones, such as, norflurazon,  
isoxazoles, such as, isoxaflutole;
- growth inhibitors, including
- 10 mitotic disruptors, of the types,  
dinitroanilines, such as, benefin, ethalfluralin, oryzalin,  
pendimethalin, prodiamine and trifluralin,  
oxysulfurons, such as, fluthiamide,  
pyridines, such as, dithiopyr and thiazopyr,
- 15 amides, such as, pronamide, and  
others, such as, DCPA;
- inhibitors of shoots of emerging seedlings, of the types,  
carbamothioates, such as, EPTC, cycloate, pebulate, triallate,  
butylate, molinate, thiobencarb and bencolate;
- 20 inhibitors of roots only of seedlings, of the types,  
amides, such as, napropamide,  
phenylureas, such as, siduron, and  
others, such as bensulide, betasan and bensumec;
- inhibitors of roots and shoots of seedlings, of the types,
- 25 chloroacetamides, such as, acetochlor, dimetenamid, propachlor,  
alachlor and metolachlor;
- inhibitors of amino acid synthesis, including,  
inhibitors of aromatic amino acid synthesis, such as, glyphosate  
and sulfosate,
- 30 inhibitors of branched chain amino acid synthesis, of the types,  
sulfonylureas, such as, bensulfuron, chlorsulfuron, halosulfuron,  
nicosulfuron, prosulfuron, fimsulfuron, thifensulfuron, tribenuron,

chlorimuron, ethametsulfuron, metsulfuron, primisulfuron, oxasulfuron,  
sulfometuron, triasulfuron and triflusulfuron,  
imidazolinones, such as, imazamethabenz, imazamox, imazapic,  
imazapyr, imazaquin and imazethapyr,  
5 triazolopyrimidines, such as, chloransulam and flumetsulam,  
tyrimidinyloxybenzoates, such as, pyriithiobac;  
lipid biosynthesis inhibitors, including,  
aryoxyphenoxypropionates, such as, ciclofop-methyl, fenoxaprop-  
ethyl, fenoxaprop-p-ethyl, fluazifop-p-butyl, haloxyfop and quizalofop-p-  
10 ethyl,  
cyclohexanediones, such as, clethodim, sethoxydim and  
tralkoxydim;  
inhibitors of cell wall biosynthesis, including,  
nitriles, such as, dichlobenil,  
15 benzamides, such as, isoxaben, and  
others, such as, quinclorac;  
cell membrane disrupters, including,  
dilute sulfuric acid, monocarbamide dihydrogen sulfate and  
herbicidal oils,  
20 bipyridyliums, such as, diquat and paraquat,  
diphenylethers, such as, acifluorfen, fomesafen, lactofen and  
oxyfluorfen,  
oxidiazoles, such as, fluthiacet and oxadiazon,  
N-phenylheterocycles, such as carfentrazone, flumiclorac and  
25 sulfentrazone;  
inhibitors of glutamine synthetase, such as glufosinate; and  
others, such as, DSMA, MSMA, asulam, endothall, ethofumesate,  
difenzoquat and TCA.  
[000100] Preferred herbicides include chlorimuron-ethyl, chloroacetic  
30 acid, chlorotoluron, chlorpropham, chlorsulfuron, chlorthal-dimethyl,  
chlorthiamid, cinmethylin, cinosulfuron, clethodim, clodinafop-propargyl,  
clomazone, clomeprop, clopyralid, cloransulam-methyl, cyanazine,

cycloate, cyclosulfamuron, cycloxydim, cyhalofop-butyl, 2,4-D, daimuron,  
 dalapon, dazomet, 2,4DB, desmedipham, desmetryn, dicamba,  
 dichlobenil, dichlorprop, dichlorprop-P, diclofop-methyl, difenzoquat  
 metilsulfate, diflufenican, dimefuron, dimepiperate, dimethachlor,  
 5 dimethametryn, dimethenamid, dimethipin, dimethylarsinic acid;  
 dinitramine, dinocap, dinoterb, diphenamid, diquat dibromide, dithiopyr,  
 diuron, DNOC, EPTC, esprocarb, ethalfluralin, ethametsulfuron-methyl,  
 ethofumesate, ethoxysulfuron, etobenzanid, fenoxaprop-P-ethyl, fenuron,  
 ferrous sulfate, flamprop-M, flazasulfuron, fluazifop-butyl, fluazifop-P-butyl,  
 10 fluchloralin, flumetsulam, flumiclorac-pentyl, flumioxazin, fluometuron,  
 fluoroglycofen-ethyl, flupoxam, flupropanate, flupyrsulfuron-methyl-sodium,  
 flurenol, fluridone, flurochloridone, fluroxypyr, flurtamone, fluthiacet-methyl,  
 fomesafen, fosamine, glufosinate-ammonium, glyphosate, halosulfuron-  
 methyl, haloxyfop, HC-252, hexazinone, imazamethabenz-methyl,  
 15 imazamox, imazapyr, imazaquin, imazethapyr, imazosuluron,  
 imidazilnone, indanofan, ioxynil, isoproturon, isouron, isoxaben,  
 isoxaflutole, lactofen, lenacil, linuron, MCPA, MCPA-thioethyl, MCPB,  
 mecoprop, mecoprop-P, mefenacet, metamitron, metazachlor,  
 methabenzthiazuron, methylarsonic acid, methylidymron, methyl  
 20 isothiocyanate, metobenzuron, metobromuron, metolachlor, metosulam,  
 metoxuron, metribuzin, metsulfuron-methyl, molinate, monolinuron,  
 naproanilide, napropamide, naptalam, neburon, nicosulfuron, nonanoic  
 acid, norflurazon, oleic acid (fatty acids), orbencarb, oryzalin, oxadiargyl,  
 oxadiazon, oxasulfuron, oxyfluorfen, paraquat dichloride, pebulate,  
 25 pendimethalin, pentachlorophenol, pentanochlor, pentoxazone, petroleum  
 oils, phenmedipham, picloram, piperophos, pretilachlor, primisulfuron-  
 methyl, prodiamine, prometon, prometryn, propachlor, propanil,  
 propaquizafop, propazine, propham, propisochlor, propyzamide,  
 prosulfocarb, prosulfuron, pyraflufen-ethyl, pyrazolynate, pyrazosulfuron-  
 30 ethyl, pyrazoxyfen, pyributicarb, pyridate, pyriminobac-methyl, pyriithiobac-  
 sodium, quinclorac, quinmerac, quinoclamine, quizalofop, quizalofop-P,  
 rimsulfuron, sethoxydim, siduron, simazine, simetryn, sodium chlorate,

STS-system, sulcotrione, sulfentrazone, sulfometuron-methyl, sulfosulfuron, sulfuric acid, tar oils, 2,3,6-TBA, TCA-sodium, tebutam, tebuthiuron, terbacil, terbumeton, terbuthylazine, terbutryn, thenylchlor, thiazopyr, thifensulfuron-methyl, thiobencarb, tiocarbazil, tralkoxydim, tri-  
allate, triasulfuron, triaziflam, tribenuron-methyl, triclopyr, trietazine,  
trifluralin, triflusulfuron-methyl, vernolate

**[000101]** Preferably, the amount of the neonicotinoid compound or other ingredients used in the seed treatment should not inhibit germination of the seed, or cause phytotoxic damage to the seed.

**[000102]** The neonicotinoid compound formulation that is used to treat the seed in the present invention can be in the form of a suspension; emulsion; slurry of particles in an aqueous medium (*e.g.*, water); wettable powder; wettable granules (dry flowable); and dry granules. If formulated as a suspension or slurry, the concentration of the neonicotinoid compound in the formulation is preferably about 0.5% to about 99% by weight (w/w), preferably 5-40%.

**[000103]** As mentioned above, other conventional inactive or inert ingredients can be incorporated into the formulation. Such inert ingredients include but are not limited to: conventional sticking agents, dispersing agents such as methylcellulose (Methocel A15LV or Methocel A15C, for example, serve as combined dispersant/sticking agents for use in seed treatments), polyvinyl alcohol (*e.g.*, Elvanol 51-05), lecithin (*e.g.*, Yelkinol P), polymeric dispersants (*e.g.*, polyvinylpyrrolidone/vinyl acetate PVP/VA S-630), thickeners (*e.g.*, clay thickeners such as Van Gel B to improve viscosity and reduce settling of particle suspensions), emulsion stabilizers, surfactants, antifreeze compounds (*e.g.*, urea), dyes, colorants, and the like. Further inert ingredients useful in the present invention can be found in McCutcheon's, vol. 1, *"Emulsifiers and Detergents,"* MC Publishing Company, Glen Rock, New Jersey, U.S.A., 1996. Additional inert ingredients useful in the present invention can be found in McCutcheon's, vol. 2, *"Functional Materials,"* MC Publishing Company, Glen Rock, New Jersey, U.S.A., 1996.

[000104] The neonicotinoid compounds and formulations of the present invention can be applied to seeds by any standard seed treatment methodology, including but not limited to mixing in a container (*e.g.*, a bottle or bag), mechanical application, tumbling, spraying, and immersion.

5 Any conventional active or inert material can be used for contacting seeds with pesticides according to the present invention, such as conventional film-coating materials including but not limited to water-based film coating materials such as Sepiret (Seppic, Inc., Fairfield, NJ) and Opacoat (Berwind Pharm. Services, Westpoint, PA).

10 [000105] The neonicotinoid compounds can be applied to a seed as a component of a seed coating. Seed coating methods and compositions that are known in the art are useful when they are modified by the addition of one of the neonicotinoid compounds of the present invention. Such coating methods and apparatus for their application are disclosed in, for example, U.S. Patent Nos. 5,918,413, 5,891,246, 5,554,445, 5,389,399, 15 5,107,787, 5,080,925, 4,759,945 and 4,465,017. Seed coating compositions are disclosed, for example, in U.S. Patent Nos. 5,939,356, 5,882,713, 5,876,739, 5,849,320, 5,834,447, 5,791,084, 5,661,103, 5,622,003, 5,580,544, 5,328,942, 5,300,127, 4,735,015, 4,634,587, 20 4,383,391, 4,372,080, 4,339,456, 4,272,417 and 4,245,432, among others.

[000106] Useful seed coatings contain one or more binders and at least one of the subject neonicotinoid compounds.

[000107] Binders that are useful in the present invention preferably comprise an adhesive polymer that may be natural or synthetic and is 25 without phytotoxic effect on the seed to be coated. The binder may be selected from polyvinyl acetates; polyvinyl acetate copolymers; ethylene vinyl acetate (EVA) copolymers; polyvinyl alcohols; polyvinyl alcohol copolymers; celluloses, including ethylcelluloses, methylcelluloses, hydroxymethylcelluloses, hydroxypropylcelluloses and 30 carboxymethylcellulose; polyvinylpyrrolidones; polysaccharides, including starch, modified starch, dextrans, maltodextrins, alginate and chitosans; fats; oils; proteins, including gelatin and zeins; gum arabics; shellacs;



vinylidene chloride and vinylidene chloride copolymers; calcium lignosulfonates; acrylic copolymers; polyvinylacrylates; polyethylene oxide; acrylamide polymers and copolymers; polyhydroxyethyl acrylate, methylacrylamide monomers; and polychloroprene.

5     **[000108]** It is preferred that the binder be selected so that it can serve as a matrix for the subject neonicotinoid compound. While the binders disclosed above may all be useful as a matrix, the specific binder will depend upon the properties of the neonicotinoid. The term "matrix", as used herein, means a continuous solid phase of one or more binder  
10     compounds throughout which is distributed as a discontinuous phase one or more of the neonicotinoid compounds. Optionally, a filler and/or other components can also be present in the matrix. The term matrix is to be understood to include what may be viewed as a matrix system, a reservoir system or a microencapsulated system. In general, a matrix system  
15     consists of a neonicotinoid compound of the present invention and filler uniformly dispersed within a polymer, while a reservoir system consists of a separate phase comprising the subject neonicotinoid compounds, that is physically dispersed within a surrounding, rate-limiting, polymeric phase. Microencapsulation includes the coating of small particles or droplets of  
20     liquid, but also to dispersions in a solid matrix.

**[000109]** The amount of binder in the coating can vary, but will be in the range of about 0.01 to about 25% of the weight of the seed, more preferably from about 0.05 to about 15%, and even more preferably from about 0.1% to about 10%.

25     **[000110]** As mentioned above, the matrix can optionally include a filler. The filler can be an absorbent or an inert filler, such as are known in the art, and may include woodflours, clays, activated carbon, sugars, diatomaceous earth, cereal flours, fine-grain inorganic solids, calcium carbonate, and the like. Clays and inorganic solids, which may be used,  
30     include calcium bentonite, kaolin, china clay, talc, perlite, mica, vermiculite, silicas, quartz powder, montmorillonite and mixtures thereof. Sugars,

which may be useful, include dextrin and maltodextrin. Cereal flours include wheat flour, oat flour and barley flour.

5 [000111] The filler is selected so that it will provide a proper microclimate for the seed, for example the filler is used to increase the loading rate of the active ingredients and to adjust the control-release of the active ingredients. The filler can aid in the production or process of coating the seed. The amount of filler can vary, but generally the weight of the filler components will be in the range of about 0.05 to about 75% of the seed weight, more preferably about 0.1 to about 50%, and even more preferably about 0.5% to 15%.

10 [000112] The amount of neonicotinoid compound that is used for the treatment of the seed will vary depending upon the type of seed and the type of neonicotinoid compound, but the treatment will comprise contacting the seeds with an amount of the neonicotinoid compound, or combination of two or more neonicotinoid compounds, that is effective for increasing the yield and/or vigor of the agronomic plant that is grown from the treated seed.

15 [000113] In general, the amount of neonicotinoid compound that is applied to a seed in the treatment will range from about 0.1 gm to about 1,000 gm of the compound per 100 kg of the weight of the seed. Preferably, the amount of neonicotinoid compound will be within the range of about 5 gm to about 600 gm active per 100 kg of seed, more preferably within the range of about 10 gm to about 400 gm active per 100 kg of seed, and even more preferably within the range of about 20 gm to about 300 gm of neonicotinoid compound per 100 kg of seed weight.

20 Alternatively, it has been found to be preferred that the amount of the neonicotinoid compound be over about 20 gm of the compound per 100 kg of the seed, and more preferably over about 40 gm per 100 kg of seed. When the neonicotinoid compound is imidacloprid, a preferred range of use includes about 40 gm/ 100 kg of seed to about 100 gm/ 100 kg.

25 [000114] \ Optionally, a plasticizer can be used in the coating formulation. Plasticizers are typically used to make the film that is formed

by the coating layer more flexible, to improve adhesion and spreadability, and to improve the speed of processing. Improved film flexibility is important to minimize chipping, breakage or flaking during storage, handling or sowing processes. Many plasticizers may be used, however, 5 useful plasticizers include polyethylene glycol, glycerol, butylbenzylphthalate, glycol benzoates and related compounds. The range of plasticizer in the coating layer will be in the range of from about 0.1 to about 20% by weight.

[000115] When the neonicotinoid compound used in the coating is an oily 10 type formulation and little or no filler is present, it may be useful to hasten the drying process by drying the formulation. This optional step may be accomplished by means well known in the art and can include the addition of calcium carbonate, kaolin or bentonite clay, perlite, diatomaceous earth, or any absorbent material that is added preferably concurrently with the 15 pesticidal coating layer to absorb the oil or excess moisture. The amount of calcium carbonate or related compounds necessary to effectively provide a dry coating will be in the range of about 0.5 to about 10% of the weight of the seed.

[000116] In a preferred embodiment, the seed coating that contains the 20 neonicotinoid compound is a controlled release coating. When the terms "controlled release" are used herein to describe a seed coating, what is meant is a seed coating that acts as a reservoir of the neonicotinoid compound and is capable of releasing the neonicotinoid compound at a rate that is slower than the neonicotinoid compound would be released if it 25 were present on the surface of the seed without being a component of a coating. Common techniques for providing controlled release coatings are described, for example, in *Controlled-Release Delivery Systems for Pesticides*, H. B. Scher, Ed., Marcel Dekker, Inc., NY (1999), and include, without limitation, matrix coatings, matrix microparticles, coated droplets, 30 coated particles, microcapsules, and the like. Some coatings formed with the neonicotinoid compound are capable of effecting a slow rate of release of the compound by diffusion or movement through the matrix to the

surrounding medium. The treated seeds may also be enveloped with a polymer film overcoating to protect the coating and/or to serve as a barrier to diffusion of the neonicotinoid compound. Such overcoatings are known in the art and may be applied using conventional fluidized bed and drum film coating techniques.

[000117] The neonicotinoid compound formulation may be applied to the seeds using conventional coating techniques and machines, such as fluidized bed techniques, the roller mill method, rotostatic seed treaters, and drum coaters. Other methods, such as spouted beds may also be useful. The seeds may be presized before coating. After coating, the seeds are typically dried and then transferred to a sizing machine for sizing. Such procedures are known in the art.

[000118] In another embodiment of the present invention, the neonicotinoid compound can be introduced onto or into a seed by use of solid matrix priming. For example, a quantity of the neonicotinoid compound can be mixed with a solid matrix material and then the seed can be placed into contact with the solid matrix material for a period to allow the neonicotinoid to be introduced to the seed. The seed can then optionally be separated from the solid matrix material and stored or used, or the mixture of solid matrix material plus seed can be stored or planted directly. Solid matrix materials which are useful in the present invention include polyacrylamide, starch, clay, silica, alumina, soil, sand, polyurea, polyacrylate, or any other material capable of absorbing or adsorbing the neonicotinoid compound for a time and releasing that compound into or onto the seed. It is useful to make sure that the neonicotinoid compound and the solid matrix material are compatible with each other. For example, the solid matrix material should be chosen so that it can release the compound at a reasonable rate, for example over a period of minutes, hours, or days.

[000119] The present invention further embodies imbibition as another method of treating seed with the neonicotinoid compound. For example, plant seed can be combined for a period of time with a solution comprising

from about 1% by weight to about 75% by weight of the neonicotinoid compound in a solvent such as water. Preferably the concentration of the solution is from about 5% by weight to about 50% by weight, more preferably from about 10% by weight to about 25% by weight. During the period that the seed is combined with the solution, the seed takes up (imbibes) a portion of the neonicotinoid compound. Optionally, the mixture of plant seed and solution can be agitated, for example by shaking, rolling, tumbling, or other means. After imbibition, the seed can be separated from the solution and optionally dried, for example by patting or air drying.

**[000120]** In yet another embodiment, a powdered neonicotinoid compound can be mixed directly with seed. Optionally, a sticking agent can be used to adhere the powder to the seed surface. For example, a quantity of seed can be mixed with a sticking agent and optionally agitated to encourage uniform coating of the seed with the sticking agent. The seed coated with the sticking agent can then be mixed with the powdered neonicotinoid compound. The mixture can be agitated, for example by tumbling, to encourage contact of the sticking agent with the powdered neonicotinoid compound, thereby causing the powdered compound to stick to the seed.

**[000121]** The treated seeds of the present invention can be used for the propagation of plants in the same manner as conventional treated seed. The treated seeds can be stored, handled, sowed and tilled in the same manner as any other pesticide treated seed. Appropriate safety measures should be taken to limit contact of the treated seed with humans, food or feed materials, water and birds and wild or domestic animals.

**[000122]** The following example describes preferred embodiments of the invention. Other embodiments within the scope of the claims herein will be apparent to one skilled in the art from consideration of the specification or practice of the invention as disclosed herein. It is intended that the specification, together with the example, be considered to be exemplary only, with the scope and spirit of the invention being indicated by the

claims which follow the examples. In the example all percentages are given on a weight basis unless otherwise indicated.

EXAMPLE 1

[000123] This example illustrates the treatment of transgenic corn seeds with imidacloprid.

[000124] Corn seeds were prepared to express *Bacillus thuringiensis* endotoxin Cry3Bb.11231 or Cry3Bb.11098 by the methods described for these respective events in WO 99/31248.

[000125] Corn seeds of the same hybrid species, with and without the respective transgenic events, were treated with imidacloprid (available as GAUCHO® from Gustafson LLC, Plano, TX) as follows. A seed treatment formulation containing imidacloprid was prepared by mixing a measured amount of the imidacloprid-containing material in water as a carrier. Also added to the mixture were other non-neonicotinoid ingredients, such as colorants, sticking agents, surfactants, lubricants, and other materials that are commonly known in the art for use in seed treatment formulations. The formulation was then applied at room temperature to a measured weight of corn seed in a rotostatic seed treater. The respective weights of the imidacloprid preparation and the corn seed were calculated to provide the desired rate of treatment of imidacloprid on the seed. The imidacloprid was mixed into sufficient water to permit efficient distribution of the formulation to all of the seeds in the batch while minimizing loss of treatment formulation due to lack of uptake of the formulation by the seeds. Treated seeds were allowed to sit uncapped for at least four hours before planting.

[000126] When the seeds were treated with imidacloprid, a sufficient amount of Gaucho® 600 FS (containing 48.7% by weight imidacloprid) was thoroughly mixed into water to form a seed treatment formulation, and the formulation was applied to a weight of corn seed via a rotostatic seed treater to provide treatment levels of 0.165 mg imidacloprid per kernel. (If one assumes that about 1650 corn kernels weigh one pound, then this

rate is equivalent to 60 grams imidacloprid per 100 kg of seed), or 1.34 mg imidacloprid per kernel (about 487 grams imidacloprid per 100 kg of seed).

#### EXAMPLE 2

[000127] This example illustrates the effect of the treatment of corn seed with imidacloprid in a hybrid seed production trial.

[000128] Twelve commercial corn hybrids were treated with imidacloprid at 0.165 mg/kernel (GAUCHO® 600). All application rates are given as the weight of the active ingredient (imidacloprid) per seed kernel. The seed treatment method was the same as described in Example 1.

[000129] The seed were planted at twenty-four trial locations across the U.S. central corn belt, using standard planting equipment. Each trial location consisted of six hybrids, with experimental treatments established where each hybrid received either a fungicide treatment alone (MAXIM® XL at 0.165 oz. active ingredient (AI)/cwt of seed), or a fungicide treatment plus a seed treatment with the neonicotinoid compound.

[000130] Trials were established as small plot (2 - 4 row by 15 - 40 feet in length) replicated experiments (four replications) in a split-plot randomized complete block design. In the experimental design, hybrids were established as main plots and seed treatments were established as sub-plots. Each trial received standard herbicide applications for weed control and other standard crop maintenance procedures, excepting that none of the trials received any additional insecticide treatments during the course of the growing season.

[000131] Experimental treatment effect was evaluated by assessing final plant stand (at growth stages V4/V5) and plot yields at harvest maturity. Plot yields were standardized at 15% moisture. The data from the trials are shown in Table 5.

**Table 5:** Yield and stand count in breeding station trials of corn hybrids which were untreated or treated with imidacloprid.

Response	Hybrid Code	Stand count (plts/ac), or Corn yield (bu/ac)			Actual Yield Over Expected	Actual Yield Over 2x of Expected
		Control	Imidacloprid treated	Difference		
Stand	A	31076	31920	843.8		
Stand	B	31622	32061	439.0		
Stand	C	30467	31217	749.5		
Stand	D	30881	31828	946.8		
Stand	E	31391	32011	620.0		
Stand	F	29624	30786	1162.3		
Stand	G	27577	28766	1189.2		
Stand	H	27964	28004	39.6		
Stand	I	26638	28460	1822.2		
Stand	J	27844	28273	429.3		
Stand	K	27961	28984	1022.8		
Stand	L	28182	28986	803.7		
Yield	A	161.5	163.5	2.0	Yes	No
Yield	B	166.7	170.8	4.1	Yes	Yes
Yield	C	153.7	159.6	5.8	Yes	Yes
Yield	D	170.1	169.4	-0.7	No	No
Yield	E	170.2	171.2	1.0	No	No
Yield	F	168.4	173.1	4.7	Yes	Yes
Yield	G	164.0	167.5	3.5	Yes	No
Yield	H	159.9	164.6	4.8	Yes	Yes
Yield	I	163.1	172.3	9.2	Yes	Yes
Yield	J	175.9	179.5	3.7	Yes	Yes
Yield	K	171.2	171.3	0.1	No	No
Yield	L	173.8	180.7	6.9	Yes	Yes

**Notes:**

- 5           a.     Key to corn hybrids is: A = DK440, B = DKC46-26, C = DK493, D = DKC51-88, E = DK537, F = LH244XLH295, G = DK567, H = HC33XLH277, I = DKC57-38, J = DK585, K = DK611, and L = RX708.
- b.     Hybrids A - F were grown at 10 locations; hybrids G - L were grown at 14 locations.
- 10          c.     Column marked "Response" shows final stand counts at V5 stage (plants/ac), and plot yields (bu/ac) at maturity.
- d.     All seeds received standard fungicide treatment.



[000132] The yield results from this set of field trials are also shown in the form of bar charts that show the increase or decrease (in bu/ac) in corn yield for seeds treated with imidacloprid as compared against untreated seeds as a function of the type of corn hybrid that was used (See Figure 2). In Figure 3, a bar chart shows the increase or decrease (in bu/ac) on corn yield for seeds treated with imidacloprid for the twenty four different locations at which the seeds were planted. Both of these charts show consistent and commercially important increases in corn yield when the seed is treated with imidacloprid irrespective of the level of insect infestation

[000133] Results from the experiments described above conclusively show the effect of imidacloprid in improving plant stand and increasing crop yield. It was notable, however, that the yield increase provided by treatment with the neonicotinoid compound in relation to the corresponding stand increases was unexpectedly high. According to publications by Purdue University, (See, e.g., Nielsen, R. L., *Estimating Yield and Dollar Returns From Corn Replanting*, AY-264-W (Rev. Jul-02), Purdue Cooperative Extension Service, West Lafayette, IN (July 2002), available at <http://www.agry.purdue.edu/ext/pubs/AY-264-W.pdf>; 4/29/03), a difference in 1,000 plants per acre in corn planted at optimal populations will provide an expected difference in yield of about 1%. (See also, Shaw, *Corn Production*, pp. 659 - 662 in, *Corn and Corn Improvement, Number 18 in the series, Agronomy*, Sprague, G. F., et al. Eds., Am. Soc. of Agronomy, Inc., Madison, WI (1988). In Table 5, the column labeled "Actual yield over expected" shows that 9 out of 12 hybrid/seed treatment combinations showed a yield increase that was more than the increase that which would have been expected on the basis of stand improvement alone as compared to the fungicide-treated control. In fact, over half of the hybrid/seed treatment combinations (7 out of 12) showed a yield increase that was over two-times that which would have been predicted on the basis of stand improvement. ("Actual yield over 2X of expected"). This

increase was unexpected and was surprisingly high. It indicated that corn yield was increased by treatment with imidacloprid, and that the increase in yield was superior to the yield increase that could be explained by increased stand counts.

5

### EXAMPLE 3

[000134] This example shows that the treatment of corn seeds with imidacloprid resulted in an increase in corn yield even at low insect pest pressure.

10 [000135] Three different corn hybrids (EXP050, EXP056, and EXP062A) were treated by the method described in Example 1 with GAUCHO® 600 FS at an application rate of 0.165 mg imidacloprid/kernel and planted at different locations. This rate of imidacloprid has been shown to not  
15 significantly reduce root injury at locations with economically damaging levels of corn rootworm, but is effective in protecting corn seedlings against stand reducing and growth limiting secondary insect pests of corn. The corn yield from an untreated control sample, from corn grown with conventional soil applied insecticides, and corn having the seed treatment of imidacloprid, were measured at harvest and are reported in Table 6.  
20 (Conventional soil-applied insecticidal treatment comprised FORCE 3G applied at 4 - 5 oz/1000 ft. of row (30 in. row spacing), LORSBAN 15G applied at the rate of 8 oz/1000 ft. of row, and COUNTER 20G applied at a rate of 6 oz/1000 ft. of row).

25

30

**Table 6:** Corn yield in field tests of non-transgenic hybrid corn with and without seed treatment with imidacloprid in areas with low pest pressure.

HYBRID	TILLAGE PRACTICE	YIELD (BU/AC)		
		UNTREATED CONTROL	SOIL APPLIED (Force 3G, Lorsban 15G, Counter 20G)	Gaucho® (0.165 mg/kernel)
EXP050	MINIMUM	134	131	140
EXP056	MINIMUM	130	130	132
EXP062A	MINIMUM	151	154	154
MEAN		138.3	138.3	142

- 5     **[000136]** Secondary insect pressure on all sites was insignificant to nonexistent. Low insect pressure was also evident from the fact that soil applied insecticides did not positively affect the yield. Treatment with imidacloprid, however, increased the yield by about 4 bu/ac (about 2.9%).
- 10    These results showed that seed treatment with imidacloprid increased corn yield, even when the level of insect pest pressure was below that which would have indicated the need for insecticide treatment.

#### EXAMPLE 4

- [000137]** This example shows that imidacloprid increases corn yield in field trials with hybrid corn with different tillage regimes.
- 15    **[000138]** Seed of three corn hybrids were treated with imidacloprid in the manner described in Example 3. Seed were planted using standard planting equipment in field experiments across the U.S. corn belt. All experiments were conducted as strip trials, under a variety of tillage regimes, and all trials received standard weed control and cultural
- 20    practices common to commercial corn production. Plot yield was collected using standard mechanical harvesting equipment.
- [000139]** Soil insecticides were applied in the manner described in Example 2. Considerable increase in corn yield was also observed when corn seed treated with imidacloprid were planted in soils treated with soil-

applied insecticide. The increase in yield was observed under all tillage conditions and for all the hybrids tested. Table 7 compares the yield from untreated corn seeds and corn seeds treated with imidacloprid when planted on soil treated with soil-applied insecticide. Seeds treated with imidacloprid produced considerably higher yield than the seeds that did not receive imidacloprid treatment. Soil-applied insecticides are known to be an effective method for controlling secondary insects, so the increase in corn yield was surprising.

**Table 7:** Yield in field trials with low insect pressure for corn having different tillage practices and with only soil insecticide treatment or soil insecticide treatment plus seed treatment with Gaucho.

TILLAGE PRACTICE <sup>a</sup>	HYBRID	YIELD (Soil applied insecticide) in Bu/Ac	YIELD (Gaucho seed treatment + Soil insecticide) in Bu/Ac
Conservation	EXP050	129	152
Conservation	EXP050	176	174
Conservation	EXP050	182	187
Conservation	EXP056	177	166
Conservation	EXP050	160	177
Conservation	EXP056	214	199
Conservation	EXP062A	219	222
MEAN		179.6	182.4
Conventional	EXP050	212	217
Conventional	EXP050	209	211
Conventional	EXP056	228	217
Conventional	EXP056	153	157
Conventional	EXP056	213	216
Conventional	EXP062A	188	185
Conventional	EXP056	182	183
Conventional	EXP056	181	192

TILLAGE PRACTICE <sup>a</sup>	HYBRID	YIELD (Soil applied insecticide) in Bu/Ac	YIELD (Gaucho seed treatment + Soil insecticide) in Bu/Ac
Conventional	EXP056	188	188
Conventional	EXP056	187	197
Conventional	EXP062A	192	201
Conventional	EXP062A	193	194
Conventional	EXP050	150	152
Conventional	EXP062A	194	207
MEAN		190.8	194.1
No Till	EXP056	105	123
No Till	EXP056	176	174
No Till	EXP056	173	172
No Till	EXP056	153	157
No Till	EXP062A	123	133
No Till	EXP062A	164	185
No Till	EXP062A	167	185
MEAN		151.8	161.3
TOTAL MEAN		178.1	183

## Notes:

a. Methods described as no-till, minimum till, conservation or conventional tillage differ from each other mainly in the degree to which the soil is disturbed prior to planting. By definition, conservation tillage leaves at least 30 percent of the soil covered by crop residues.

No-Till - Tillage system refers to situations where no tillage methods are applied to the soil prior to or at planting.

Conservation/Minimum tillage refers to reduced tillage practices allowing at least 30% of soil surface to remain covered with crop residue.

Conventional Tillage refers to tillage practices where less than 30% of soil surface is covered with previous crop residue.

Such practices may include moldboard plowing, disking, or multiple field cultivation passes prior to or at planting of crop. Strip and Ridge-Till, although usually grouped in conservation tillage because of beneficial effects on soil and water retention, are grouped in conventional tillage here because the tilled zones in which the crop is planted provide an environment more similar to true conventional tillage.

[000140] The data from this trial also indicated that the yield of corn grown with the use of no-till cultivation was increased by a higher percentage (increase of 6.3%) than corn receiving convention tillage (increase of 1.7%), or conservation/minimum tillage (increase of 1.6%).

#### EXAMPLE 5

[000141] This example shows the effect on yield of treating isoline and transgenic corn seed with imidacloprid.

[000142] Corn hybrids with and without transgenic events expressing insecticidal proteins were evaluated for field efficacy. The hybrids that were evaluated were RX670 and RX601, each as an isoline (having no transgenic events) and each having a transgenic event expressing the Cry3Bb protein having activity against *Diabrotica spp.* (corn rootworm) -- designated as MON853, or a transgenic event expressing the Cry3Bb protein having activity against corn rootworm -- designated as MON863, or a combination of MON853 and a transgenic event expressing the Cry1A protein having activity against European corn borer -- designated as MON810, or a combination of MON863 and MON810. Accordingly, an isoline and four transgenic forms were tested for each hybrid. Each type of seed was tested with and without seed treatment with 60 gm of imidacloprid per 100 kg of seed, applied in a Niklas seed treater. The isoline seeds were also tested with and without the use of conventional soil-applied insecticides, which were applied as described in Example 3.

[000143] Corn yield for the tests is shown in Tables 8 and 9.

**Table 8:** Corn yield in field tests of non-transgenic hybrid corn with and without seed treatment with imidacloprid in areas with low pest pressure.

LOCATION	HYBRID	YIELD (BU/AC)		
		UNTREATED CONTROL and (Root Damage Rating) <sup>a</sup>	SOIL APPLIED (Force 3G, Lorsban, Counter) <sup>b</sup>	Gaucha (60g/100 kg seed)
1	RX670	76.8 (RDR = 2.3)	74.8	73
2	RX601	105.1 (RDR = 1.9)	98.7	101.5
3	RX601	199.3 (RDR = 2.5)	171.1	180
4	RX601	119.8 (RDR = 2.6)	117.1	117.1

5 **Notes:**

a. Root Damage Rating (RDR) is measured according to the Iowa Root Rating system (Hill and Peters, 1971, *ibid.*) and is expressed on a scale of from 0 - 6. Any score below 3 indicates a damage level that would be considered to indicate that no insecticide treatment was required.

10 b. Seed treatment with Force 3G, Lorsban 15G, and Counter 20G, was carried out according to the practice and at the rates described in Example 3.

15 [000144] The data shown in Table 8 indicated that the yield for isoline corn was increased by imidacloprid treatment in one-half of the cases tested. The increases, however, were not substantial. The data also showed that root damage pressure due to corn rootworm during the tests was not significant.

20 [000145] In contrast, however, when imidacloprid was applied to transgenic seeds in this test, the increase in corn yield over that obtained from untreated transgenic seed was substantial. Table 9 shows that in all of the trials, transgenic seed treated with imidacloprid yielded higher than

the isolines, and also higher than the untreated transgenic strains. In the relative absence of insect pressure, this result was unexpected.



**Table 9:** Corn yield in field trials having low pest pressure for corn seed having transgenic events giving corn root worm and European Corn Borer protection.

LOCATION AND HYBRID	YIELD (BU/AC)									
	UNTREATED CONTROL (Root Damage Rating)	HYBRID 853	HYBRID 853 + Gaucho	HYBRID 863	HYBRID 863 + Gaucho	HYBRID 853/810	HYBRID 853/810 + Gaucho	HYBRID 863/810	HYBRID 863/810 + Gaucho	HYBRID
1 RX(670)	76.8 (RDR=2.3)	52.5	78	70.7	85.8		91.1		93.3	
2 (RX601)	105.1 (RDR=1.9)					102.6	107.4	111.6	115.3	
3 (RX601)	199.3 (RDR=2.5)		187.1		208.9					
4 (RX601)	117.1 (RDR=2.6)						114.3		118.7	

### EXAMPLE 6

[000146] This example illustrates how the United States crop reporting district tables for insecticide use in corn can be used to determine whether insect pressure in a particular location indicates a need for treatment with an insecticide.

[000147] Referring to Table 1, a person selects a location to plant corn within U.S. crop reporting district (CRD) no. 01050. From Table 1, it can be seen that in 2001, no insecticide treatment of corn is reported on corn acreage in that CRD. Accordingly, it can be determined that the level of insect pressure at that location is below that at which treatment of the corn seed with an insecticide would be indicated.

[000148] By consulting Table 1, similar conclusions may be drawn for CRD's 01010, 01020, 01030, 01060, 04020, 04050, 05050, 05070, 06060, 13020, 16090, 20050, 22010, 22040, 22060, 22070, 23010, 23020, 26010, 26020, 26030, 27020, 27030, 28020, 28040, 28070, 28090, 29080, 30030, 30080, 30090, 32010, 33010, 34080, 37040, 38010, 38020, 38030, 38040, 38050, 38070, 38080, 40020, 40030, 40040, 40050, 40060, 40070, 40080, 40090, 44010, 45010, 45040, 46020, 46040, 46050, 46070, 46080, 48021, 49060, 53010, 53020, 53090, 55020, and 55030.

### EXAMPLE 7

[000149] This example illustrates how records on insecticide use on corn by county can be used to determine whether insect pressure in a particular location indicates a need for treatment with an insecticide.

[000150] If a person selects a location to plant corn in a county within a U.S. crop reporting district (CRD), where some level of insecticide use in the CRD is indicated -- for example in CRD number 01040, but the county in which the person elects to plant corn is known to be free of insecticide use on corn, then it can be determined that the level of insect pressure at such county is below that at which treatment of the corn seed with an insecticide would be indicated.

[000151] A similar conclusion may be drawn for every county for which the level of insecticide use on corn can be determined.

5      **[000152]** All references cited in this specification, including without limitation all papers, publications, patents, patent applications, presentations, texts, reports, manuscripts, brochures, books, internet postings, journal articles, periodicals, and the like, are hereby incorporated by reference into this specification in their entireties. The discussion of the references herein is intended merely to summarize the assertions made by their authors and no admission is made that any reference constitutes prior art. Applicants reserve the right to challenge the accuracy and pertinency of the cited references.

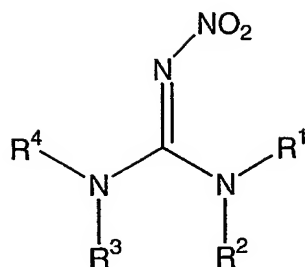
10      **[000153]** In view of the above, it will be seen that the several advantages of the invention are achieved and other advantageous results obtained.

15      **[000154]** As various changes could be made in the above methods and compositions without departing from the scope of the invention, it is intended that all matter contained in the above description and shown in the accompanying drawings shall be interpreted as illustrative and not in a limiting sense.

**WHAT IS CLAIMED IS:**

1. A method of increasing the yield and/or vigor of an agronomic plant that is grown from a seed, the method comprising:
  - a. determining whether the seed is to be planted in a location having a level of insect pest infestation that would indicate treatment with an insecticide; and, if such treatment is not indicated,
  - b. carrying out an action that is selected from the group consisting of:
    - i. treating the seed with a neonicotinoid compound,
    - ii. recommending the purchase of a seed that has been treated with a neonicotinoid compound for planting in the location,
    - iii. selling a seed that has been treated with a neonicotinoid compound for planting in the location, and
    - iv. planting in the location a seed that has been treated with a neonicotinoid compound.
2. A method of increasing the yield and/or vigor of an agronomic plant that is grown from a seed that is planted in a location having a level of infestation by an insect that is a pest for the agronomic plant and against which a neonicotinoid compound has insecticidal activity, the method comprising:
  - a. determining whether the level of infestation by the insect that is a pest for the agronomic plant indicates treatment with an insecticide; and, if treatment is not indicated,
  - b. treating the seed with a neonicotinoid compound.
3. The method according to claim 2, comprising:
  - a. comparing a level of infestation by the insect at the location with a level of infestation by the insect at which treatment with an insecticide would be indicated; and, if the level of infestation of the location by the insect is lower than the level of infestation at which treatment is indicated,
  - b. treating the seed with a neonicotinoid compound.
4. The method according to claim 2, comprising:

- a. determining the level of infestation by the insect at the location;
  - b. determining a level of infestation by the insect at which treatment with an insecticide would be indicated;
  - c. comparing the level of infestation by the insect at the location with the level of infestation by the insect at which treatment with an insecticide would be indicated; and, if the level of infestation of the location by the insect is lower than the level of infestation at which treatment is indicated,
  - d. treating the seed with a neonicotinoid compound.
5. The method according to claim 2, comprising:
- a. determining whether the level of infestation by the insect that is a pest for the agronomic plant indicates that treatment with an insecticide is needed; and, if treatment is not indicated,
  - b. planting in the location a seed that has been treated with a neonicotinoid compound.
6. The method according to claim 2, comprising:
- a. determining the level of infestation by the insect at the location;
  - b. determining a level of infestation by the insect at which treatment with an insecticide would be indicated;
  - c. comparing the level of infestation by the insect at the location with the level of infestation by the insect at which treatment with an insecticide would be indicated; and, if the level of infestation of the location by the insect is lower than the level of infestation at which treatment is indicated,
  - d. planting the seed after it has been treated with a neonicotinoid compound.
7. The method according to any one of claims 1 through 6, wherein the neonicotinoid compound comprises a compound having the formula:



where:

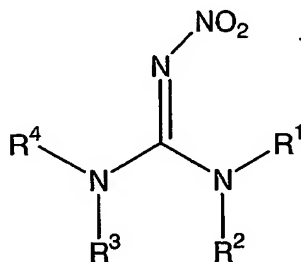
$R^1$  is hydrogen, or  $C_1 - C_4$  alkyl;

$R^2$  is hydrogen,  $C_1 - C_4$  alkyl,  $C_1 - C_4$  alkenyl,  $C_1 - C_4$  alkynyl, hydroxyl, amino, aryl, thio, alkylaryl, arylalkyl, or  $C_4 - C_6$  heterocyclic;

$R^3$  is hydrogen,  $C_1 - C_4$  alkyl,  $C_1 - C_4$  alkenyl,  $C_1 - C_4$  alkynyl, hydroxyl, amino, aryl, thio, alkylaryl, arylalkyl, or 4 - 6-member heterocyclic; or is such that  $R^2$  and  $R^3$  can join to form a 4 - 6 member heterocyclic, which may optionally be substituted or unsubstituted; and

$R^4$ , if present, is hydrogen,  $C_1 - C_4$  alkyl,  $C_1 - C_4$  alkenyl,  $C_1 - C_4$  alkynyl, hydroxyl, amino, aryl, thio, alkylaryl, arylalkyl,  $C_4 - C_6$  heterocyclic, halothiazoylalkyl, or furylalkyl.

8. The method according to any one of claims 1 through 6, wherein the neonicotinoid compound comprises a compound having the



formula:

where:

$R^1$  is hydrogen, or methyl;

$R^2$  is hydrogen, or methyl;

$R^3$  is hydrogen, methyl, or of a form that can join with  $R^2$  to form an oxadiazine ring or a 2,3-diazol ring; and

$R^4$ , if present, is chlorothiazoylmethyl, or furylmethyl.

9. The method according to any one of claims 1 through 6, wherein the neonicotinoid compound is selected from the group consisting of acetamiprid, imidacloprid, thiamethoxam, clothianidin, dinotefuran, nitenpyram, flonicamid, nithiazine and thiacloprid.

10. The method according to claim 9, wherein the neonicotinoid compound is selected from the group consisting of acetamiprid, imidacloprid, thiamethoxam, clothianidin, dinotefuran and nitenpyram.

11. The method according to claim 7, wherein the seed comprises a foreign polynucleotide sequence encoding for the production of an insecticidal protein.

12. The method according to claim 11, wherein the seed comprises a foreign polynucleotide sequence encoding a modified *B. thuringiensis*  $\delta$ -endotoxin.

13. The method according to claim 12, wherein the modified  $\delta$ -endotoxin is one that is expressed by the foreign *B. thuringiensis* gene sequence that is present in a strain selected from the group consisting of strains having deposit numbers NRRL B-21579, NRRL B-21580, NRRL B-21581, NRRL B-21635, and NRRL B-21636.

14. The method according to claim 12, wherein the modified  $\delta$ -endotoxin is one that is expressed by the foreign *B. thuringiensis* gene sequence that is present in a strain selected from the group consisting of strains having deposit numbers NRRL B-21744, NRRL B-21745, NRRL B-21746, NRRL B-21747, NRRL B-21748, NRRL B-21749, NRRL B-21750, NRRL B-21751, NRRL B-21752, NRRL B-21753, NRRL B-21754, NRRL B-21755, NRRL B-21756, NRRL B-21757, NRRL B-21758, NRRL B-21759, NRRL B-21760, NRRL B-21761, NRRL B-21762, NRRL B-21763, NRRL B-21764, NRRL B-21765, NRRL B-21766, NRRL B-21767, NRRL B-21768, NRRL B-21769, NRRL B-21770, NRRL B-21771, NRRL B-21772,

NRRL B-21773, NRRL B-21774, NRRL B-21775, NRRL B-21776, NRRL B-21777, NRRL B-21778, and NRRL B-21779.

15. The method according to claim 12, wherein the modified  $\delta$ -endotoxin is selected from the group consisting of Cry3Bb.11230, Cry3Bb.11231, Cry3Bb.11232, Cry3Bb.11233, Cry3Bb.11234, Cry3Bb.11235, Cry3Bb.11236, Cry3Bb.11237, Cry3Bb.11238, Cry3Bb.11239, Cry3Bb.11241, Cry3Bb.11242, Cry3Bb.11098, a binary insecticidal protein CryET33 and CryET34, a binary insecticidal protein CryET80 and CryET76, a binary insecticidal protein tIC100 and tIC101, and a binary insecticidal protein PS149B1.

16. The method according any one of claims 1 through 6, having the added step of treating the soil in which the seed is planted with the neonicotinoid compound.

17. The method according to any one of claims 1 through 6, having the additional step of cultivating the seed and the plant which grows from the seed according to no-till practice.

18. The method according to claim 1, wherein determining whether the seed is to be planted in a location having a level of insect pest infestation that would indicate the need for treatment with an insecticide comprises determining whether the seed is to be planted in a location having a level of insect pest infestation that would indicate the need for treatment with a neonicotinoid insecticide.

19. The method according to any one of claims 7, 10, and 11, wherein the seed is treated with an amount of the neonicotinoid compound from about 0.1 gm/100 kg of seed to about 1,000 gm/100kg of seed.

20. The method according to claim 19, wherein the seed is treated with a neonicotinoid compound in an amount of from about 5 gm/100 kg of seed to about 600 gm/100 kg of seed.

21. The method according to claim 20, wherein the seed is treated with a neonicotinoid compound in an amount of from about 10 gm/100 kg of seed to about 400 gm/100 kg of seed.



22. The method according to claim 21, wherein the seed is treated with a neonicotinoid compound in an amount of from about 20 gm/100 kg of seed to about 300 gm/100 kg of seed.

23. The method according to any one of claims 7, 10, and 11, wherein the agronomic plant is selected from the group consisting of cereals, wheat, barley, rye, oats, rice, sorghum, beet, pear-like fruits, stone fruits, soft fruits, apple, pear, plum, peach, Japanese apricot, prune, almond, cherry, strawberry, raspberry, black berry, legumes, kidney bean, lentil, pea, soybean, oil plants, rape, mustard, poppy, olive, sunflower, coconut, castor-oil plant, cocoa bean, peanut, Cucurbitaceae, pumpkin, cucumber, melon, citrus, orange, lemon, grape fruit, mandarin, Watson pomelo, citrus natsudaidai, vegetables, lettuce, cabbage, celery cabbage, Chinese radish, carrot, onion, tomato, potato, green pepper, camphor trees, avocado, cinnamon, camphor, corn, tobacco, nuts, coffee, sugar cane, tea, grapevine, hop and banana.

24. The method according to any one of claims 7, 10, and 11, wherein the agronomic plant is selected from the group consisting of rice, wheat, barley, rye, corn, potato, carrot, sweet potato, sugar beet, bean, pea, chicory, lettuce, cabbage, cauliflower, broccoli, turnip, radish, spinach, asparagus, onion, garlic, eggplant, pepper, celery, canot, squash, pumpkin, zucchini, cucumber, apple, pear, quince, melon, plum, cherry, peach, nectarine, apricot, strawberry, grape, raspberry, blackberry, pineapple, avocado, papaya, mango, banana, soybean, tomato, sorghum and raspberries and banana.

25. The method according to any one of claims 7, 10, and 11, wherein the agronomic plant is selected from the group consisting of cotton, flax, hemp, jute, ramie, sisal, pine, oak, redwood, poplar, gum, ash, fir, birch, hemlock, larch, mahogany, ebony, ornamental shrubs, and ornamental trees.

26. The method according to any one of claims 7, 10, and 11, wherein the agronomic plant is selected from the group consisting of corn, cereals, barley, rye, rice, vegetables, clovers, legumes, beans, peas,

alfalfa, sugar cane, sugar beets, tobacco, cotton, rapeseed (canola), sunflower, safflower, and sorghum.

27. The method according to claim 26, wherein the agronomic plant is corn.

28. The method according to claim 26, wherein the agronomic plant is a soybean plant.

29. The method according to any one of claims 7, 10, and 11, wherein the treatment of the seed of the plant comprises, in addition, treatment of the seed with a fungicide selected from the group consisting of fludioxonil, fluquinconazole, difenoconazole, captan, metalaxyl, carboxin, azoxystrobin, ipconazole, and thiram.

30. The method according to any one of claims 7, 10, and 11, wherein the seed possesses a transgenic event providing the plant with resistance to a herbicide and the treatment comprises foliar application of the herbicide.

31. The method according to claim 30, wherein the herbicide is selected from the group consisting of growth regulators, phenoxy acetic acids, phenoxy propionic acids, phenoxy butyric acids, benzoic acids, picolinic acid and related compounds, clopyralid, quinclorac, inhibitors of auxin transport, semicarbones, s-triazines, other triazines, substituted ureas, uracils, benzothiadiazoles, benzonitroles, phenylcarbamates, pyridazinones, phenypyridazines, pigment inhibitors, pyridazinones, isoxazoles, growth inhibitors, mitotic disruptors, dinitroanilines, oxysulfurons, pyridines, amides, inhibitors of shoots of emerging seedlings, carbamothioates, inhibitors of roots only of seedlings, amides, phenylureas, inhibitors of roots and shoots of seedlings, chloroacetamides, inhibitors of aromatic amino acid synthesis, inhibitors of branched chain amino acid synthesis, sulfonyleureas, midazolinones, triazolopyrimidines, tyrimidinyloxybenzoates, lipid biosynthesis inhibitors, aryoxyphenoxypropionates, cyclohexanediones, inhibitors of cell wall biosynthesis, nitriles, benzamides, cell membrane disrupters, dilute sulfuric acid, monocarbamide dihydrogen sulfate, herbicidal oils, bipyridyliums,

diphenylethers, oxidiazoles, N-phenylheterocycles, and inhibitors of glutamine synthetase.

32. The method according to claim 30, wherein the herbicide is selected from the group consisting of chlorimuron-ethyl, chloroacetic acid, chlorotoluron, chlorpropham, chlorsulfuron, chlorthal-dimethyl, chlorthiamid, cinmethylin, cinosulfuron, clethodim, clodinafop-propargyl, clomazone, clomeprop, clopyralid, cloransulam-methyl, cyanazine, cycloate, cyclosulfamuron, cycloxydim, cyhalofop-butyl, 2,4-D, daimuron, dalapon, dazomet, 2,4DB, desmedipham, desmetryn, dicamba, dichlobenil, dichlorprop, dichlorprop-P, diclofop-methyl, difenzoquat metilsulfate, diflufenican, dimefuron, dimepiperate, dimethachlor, dimethametryn, dimethenamid, dimethipin, dimethylarsinic acid, dinitramine, dinocap, dinoterb, diphenamid, diquat dibromide, dithiopyr, diuron, DNOC, EPTC, esprocarb, ethalfluralin, ethametsulfuron-methyl, ethofumesate, ethoxysulfuron, etobenzanid, fenoxaprop-P-ethyl, fenuron, ferrous sulfate, flamprop-M, flazasulfuron, fluazifop-butyl, fluazifop-P-butyl, fluchloralin, flumetsulam, flumiclorac-pentyl, flumioxazin, fluometuron, fluoroglycofen-ethyl, flupoxam, flupropanate, flupyrsulfuron-methyl-sodium, flurenol, fluridone, flurochloridone, fluroxypyr, flurtamone, fluthiacet-methyl, fomesafen, fosamine, glufosinate-ammonium, glyphosate, halosulfuron-methyl, haloxyfop, HC-252, hexazinone, imazamethabenz-methyl, imazamox, imazapyr, imazaquin, imazethapyr, imazosuluron, imidazilnone, indanofan, ioxynil, isoproturon, isouron, isoxaben, isoxaflutole, lactofen, lenacil, linuron, MCPA, MCPA-thioethyl, MCPB, mecoprop, mecoprop-P, mefenacet, metamitron, metazachlor, methabenzthiazuron, methylarsonic acid, methylsulfuron, methyl isothiocyanate, metobenzuron, metobromuron, metolachlor, metosulam, metoxuron, metribuzin, metsulfuron-methyl, molinate, monolinuron, naproanilide, napropamide, naptalam, neburon, nicosulfuron, nonanoic acid, norflurazon, oleic acid (fatty acids), orbencarb, oryzalin, oxadiargyl, oxadiazon, oxasulfuron, oxyfluorfen, paraquat dichloride, pebulate, pendimethalin, pentachlorophenol, pentanochlor, pentoxazone, petroleum

oils, phenmedipham, picloram, piperophos, pretilachlor, primisulfuron-methyl, prodiamine, prometon, prometryn, propachlor, propanil, propaquizafop, propazine, propham, propisochlor, propyzamide, prosulfocarb, prosulfuron, pyraflufen-ethyl, pyrazolynate, pyrazosulfuron-ethyl, pyrazoxyfen, pyributicarb, pyridate, pyriminobac-methyl, pyriothiobac-sodium, quinclorac, quinmerac, quinochloramine, quizalofop, quizalofop-P, rimsulfuron, sethoxydim, siduron, simazine, simetryn, sodium chlorate, STS-system, sulcotrione, sulfentrazone, sulfometuron-methyl, sulfosulfuron, sulfuric acid, tar oils, 2,3,6-TBA, TCA-sodium, tebutam, tebuthiuron, terbacil, terbumeton, terbuthylazine, terbutryn, thenylchlor, thiazopyr, thifensulfuron-methyl, thiobencarb, tiocarbazil, tralkoxydim, triallate, triasulfuron, triaziflam, tribenuron-methyl, triclopyr, trietazine, trifluralin, triflusulfuron-methyl, vernolate, and mixtures thereof.

33. The method according to claim 30, wherein the herbicide is selected from the group consisting of glyphosate, glyfosinate, glufosinate, imidazilinone and STS system.

34. The method according to claim 33, wherein the herbicide comprises glyphosate.

35. The method according to claim 33, wherein the modified  $\delta$ -endotoxin is selected from Cry3Bb 11231 and Cry3Bb 11098.

36. The method according to any one of claims 7, 10, and 11, wherein the seed is treated with a neonicotinoid compound which is a component of a controlled release coating.

37. A method of breeding a hybrid plant having increased yield and/or vigor from two parent plants, the method comprising:

treating the seeds of one or both of the parent plants with a neonicotinoid compound prior to planting the seeds;

pollinating the female parent with pollen of the male parent; and

gathering the seed produced by the female parent plant.

38. The method of breeding according to claim 37, wherein one or both of the parent plants contain a foreign gene that encodes for the production of a pesticidal protein.

39. The method according to claim 38, wherein the pesticidal protein comprises an insect toxin.

40. The method according to claim 39, wherein the insect toxin is a *Bacillus thuringiensis* delta-endotoxin.

41. The method according to claim 39, wherein the insect toxin is a modified *B. thuringiensis* delta-endotoxin of the type that is described in claim 15.

42. A method of increasing the yield and/or vigor of an agronomic plant that is grown from a seed that is planted in a location where treatment of the seed or the agronomic plant with an insecticide is not indicated, the method comprising treating a seed with a neonicotinoid compound and planting the treated seed in a location where treatment of the seed or the agronomic plant with an insecticide is not practiced.

43. A method of increasing the yield and/or vigor of an agronomic plant that is grown from a seed that is planted in a location having a level of infestation by an insect that is a pest for the agronomic plant and against which a neonicotinoid insecticide has insecticidal activity, the method comprising treating a seed with a neonicotinoid compound and planting the treated seed in a location where insecticide treatment of the seed or the agronomic plant is not practiced.

44. The method according to any one of claims 42 and 43, wherein planting the treated seed in a location where treatment of the seed or the agronomic plant with an insecticide is not practiced comprises planting the seed in a crop reporting district in which no insecticide was used on the crop during the previous year.

45. The method according to claim 44, wherein planting the treated seed in a location where treatment of the seed or the agronomic plant with an insecticide is not practiced comprises planting the seed in a crop reporting district in which no neonicotinoid insecticide was used on the crop during the previous year.

46. The method according to claim 42, wherein planting the treated seed in a location where treatment of the seed or the agronomic

plant with an insecticide is not practiced comprises planting the seed in a county in which no insecticide was used on the crop during the previous year.

47. The method according to claim 46, wherein planting the treated seed in a location where treatment of the seed or the agronomic plant with an insecticide is not practiced comprises planting the seed in a county in which no neonicotinoid insecticide was used on the crop during the previous year.

48. A method of increasing the yield and/or vigor of an agronomic plant that is grown from a seed that is planted in a location having a level of infestation by an insect that is a pest for the agronomic plant and against which a neonicotinoid insecticide has insecticidal activity, the method comprising:

- a. treating a seed with a neonicotinoid insecticide; and
- b. planting the treated seed in a location having a level of insect infestation below that at which such insecticide treatment is indicated.

49. A method of marketing plant seed that are treated with a neonicotinoid compound to provide an increase in the yield and/or vigor of an agronomic plant that is grown from the seed, the method comprising:

- a. determining whether the seed is to be planted in a location having a level of insect infestation that indicates a need for such treatment, and, if not;
- b. carrying out an action selected from the group consisting of:
  - i. recommending that such treated seed be purchased and planted,
  - ii. advertising such treated seed,
  - iii. obtaining such treated seed for resale, and
  - iv. selling such treated seed.

50. The method according to claim 49, wherein the action comprises advertising such treated seed, wherein the advertisement describes the property of the treated seed of providing an increase in the yield and/or vigor of an agronomic plant that is grown from the seed.

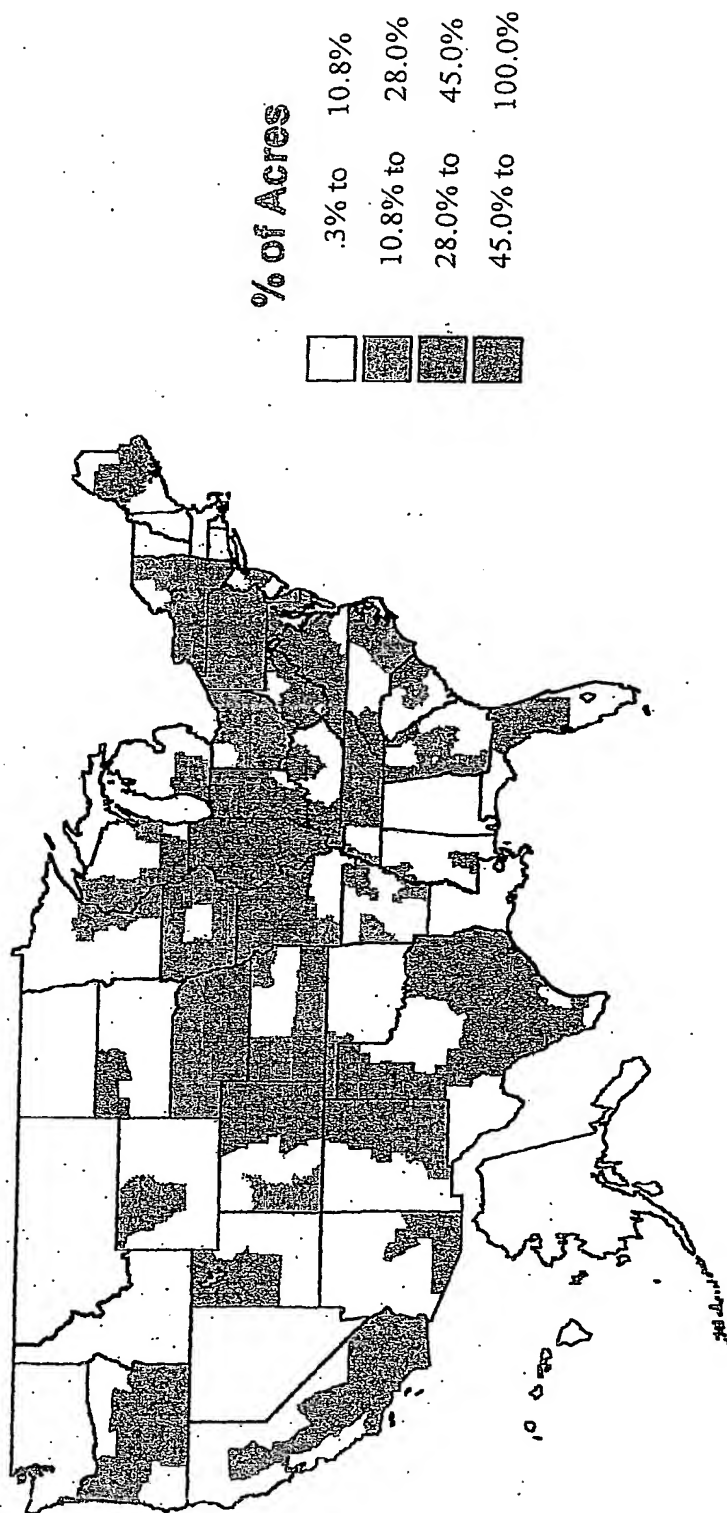
51. A seed that is treated by the method according to any one of claims 7, 10, and 11.

52. A method of increasing the yield and/or vigor of an agronomic plant that is grown from a seed, the method comprising:

a. selecting a location in which the seed is to be planted where the level of insect pest infestation is below that at which treatment with an insecticide is indicated; and

b. carrying out an action that is selected from the group consisting of:

- i. treating the seed with a neonicotinoid compound,
- ii. recommending the purchase of a seed that has been treated with a neonicotinoid compound for planting in the location,
- iii. selling a seed that has been treated with a neonicotinoid compound for planting in the location, and
- iv. planting in the location a seed that has been treated with a neonicotinoid compound.



**FIG. 1**



FIGURE 2.

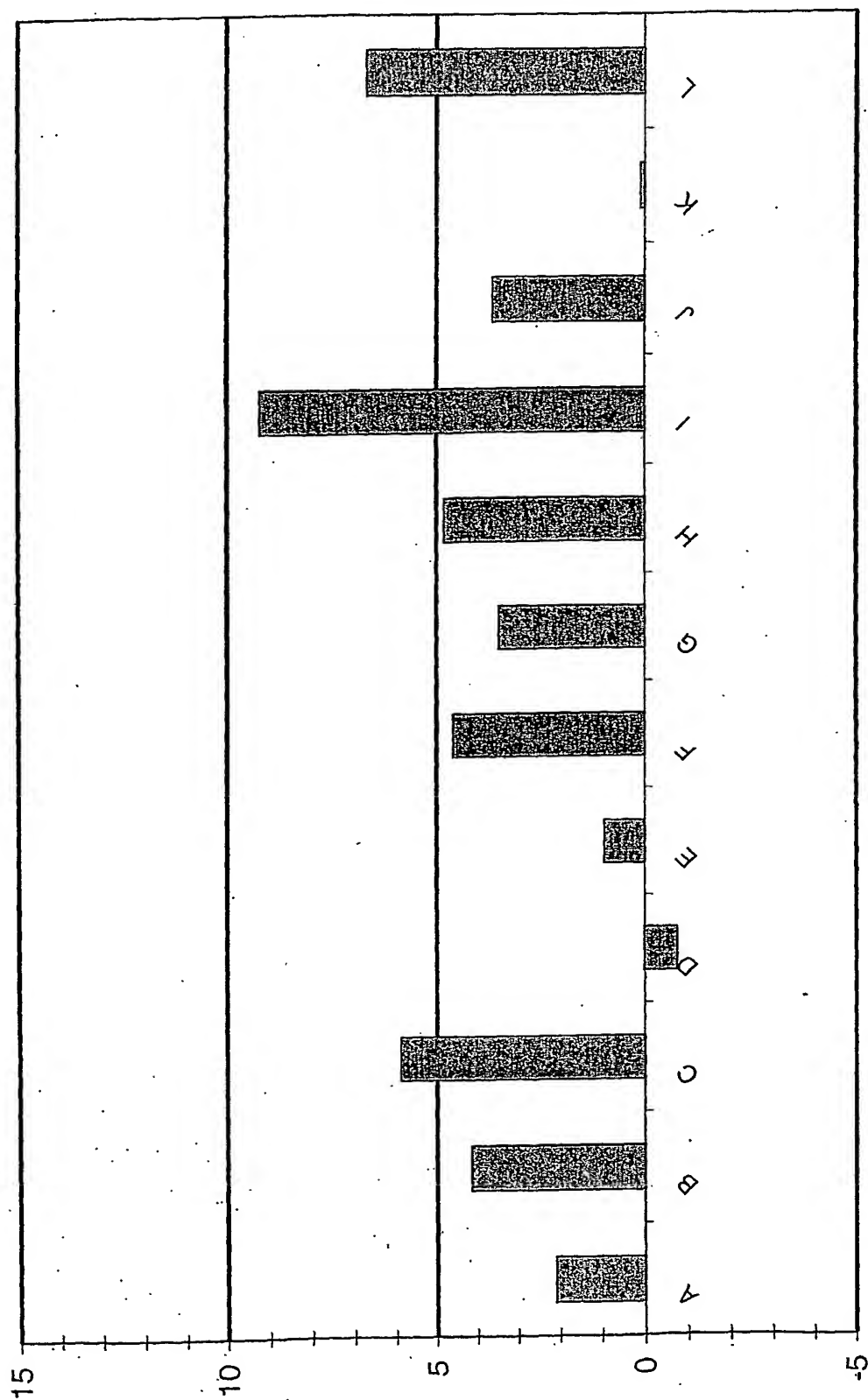
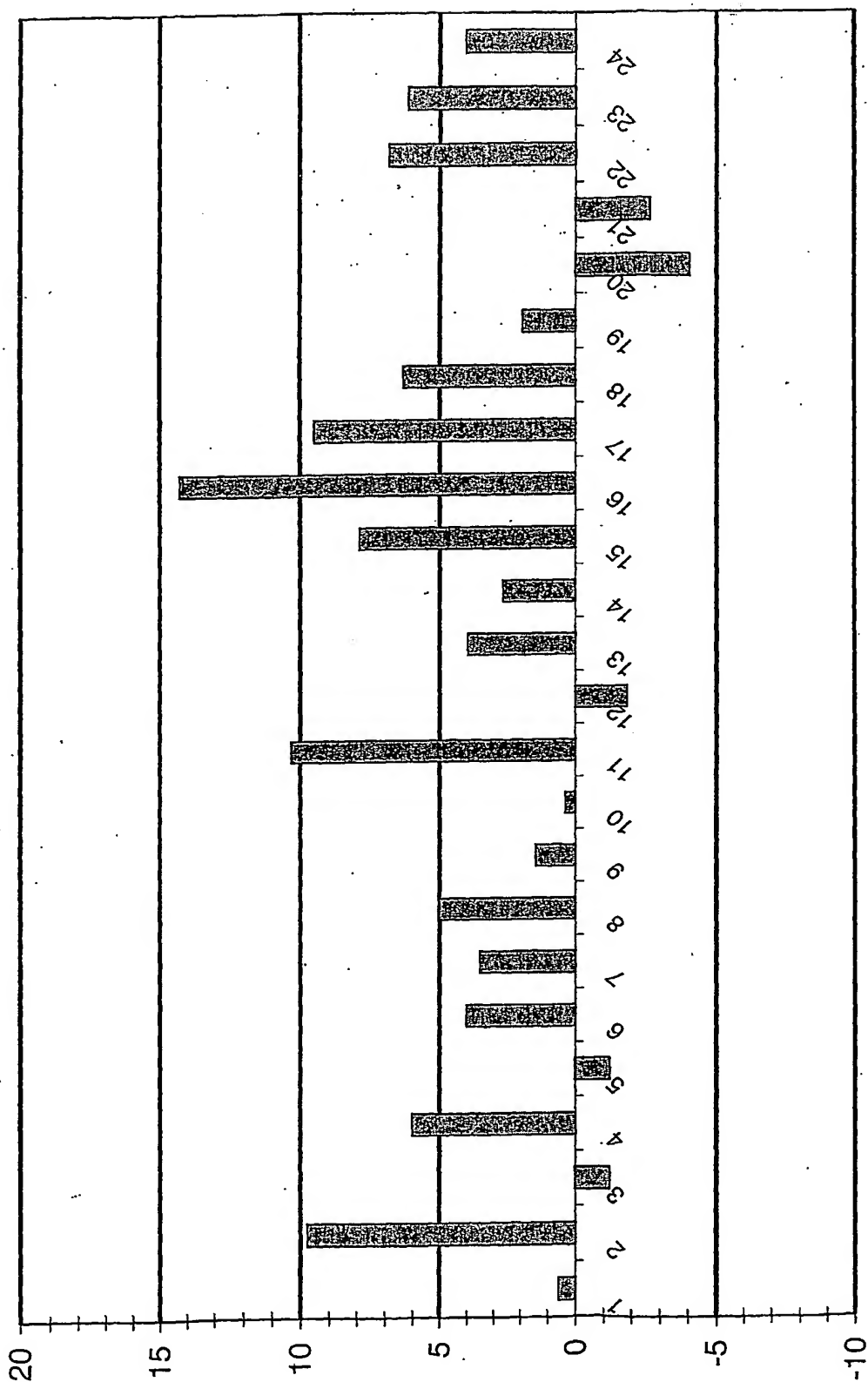


FIGURE 3.



## INTERNATIONAL SEARCH REPORT

 Internat<sup>l</sup> application No  
 PCT/US 03/15249

 A. CLASSIFICATION OF SUBJECT MATTER  
 IPC 7 A01N51/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

WPI Data, EPO-Internal, CHEM ABS Data

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 01 26468 A (SYNGENTA PARTICIPATIONS) 19 April 2001 (2001-04-19) page 1, line 1 - line 3 page 1, line 11 - line 14 page 3, line 5 - line 19 page 4, line 3 - line 19 page 8, line 9 - line 13 examples B1-B4	1-48, 51, 52

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

## \* Special categories of cited documents:

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the International filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

\*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

\*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

\*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

\*Z\* document member of the same patent family

Date of the actual completion of the international search

4 September 2003

Date of mailing of the international search report

15/09/2003

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Authorized officer

Fort, M

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US 03/15249**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 1 (partially), 49-50, 52 (partially)  
because they relate to subject matter not required to be searched by this Authority, namely:  
methods for doing business (rule 39.1 (iii) PCT).
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

on patent family members

Internatic Application No

PCT/US 03/15249

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 0126468	A	19-04-2001	AU 2834301 A	23-04-2001
			BR 0014763 A	11-06-2002
			EG 22688 A	30-06-2003
			WO 0126468 A2	19-04-2001
			EP 1227726 A2	07-08-2002

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